TITLE PAGE

Functional Polymers For Biomedical Application.

Synthesis and Applications

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Doctor of Philosophy

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A thesis submitted for the degree of Doctor of Philosophy by

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SUMMARY

Aromatic and aliphatic diacid chlorides were used to condense naturally occurring diamino acids and their esterified derivatives. It was anticipated the resulting functional polyamides would biodegrade to physiologically acceptable compounds and show pH dependant solubility could be used for biomedical applications ranging from enteric coatings to hydrosoluble drug delivery vehicles capable of targeting areas of low physiological pH. With these applications in mind the polymers were characterised by infra red spectroscopy, gel permeation chromatography and in the case of aqueous soluble polymers by potentiometric titration. Thin films of poly (lysine ethyl ester *iso*-phthalamide) plasticised with poly (caprolactone) were cast from DMSO/chloroform solutions and their mechanical properties measured on a Hounsfield Hti tensiometer.

Interfacial synthesis was investigated as a synthetic route for the production of linear functional polyamides. High molecular weight polymer was obtained only when esterified diamino acids were condensed with aromatic diacid chlorides. The method was unsuitable for the production of copolymers of free and esterified amino acids with a diacid chloride.

A novel miscible mixed solvent single phase reaction was investigated for production of copolymers of esterified and non-esterified amino acids with diacid chlorides. Aliphatic diacid chlorides were unsuitable for condensing diamino acids using this technique because of high rates of hydrolysis. The technique gave high molecular weight homopolymers from esterified diamino acids and aromatic diacid chlorides.

Two Carbon atoms were walking down the street one day. One carbon atom suddenly turned to his friend and said "Oh No! I think I've lost an electron." " Are you sure?" inquired the other "Yes I'm positive." replied the first.

(Source Colin from lab 307)

DEDICATION

I dedicate this work to my Mother and Father and to the memory of my Grandfather and Grandmother.

ACKNOWLEDGEMENTS

I would like to offer my gratitude to Professor Brian Tighe for his encouragement and advice throughout this work.

I also wish to thank my girlfriend Louise Cooke who has been extremely supportive and patient whilst I have squandered my time and attention on the completion of this thesis. In the final weeks Louise has been a source of encouragement providing much needed distraction at times thus preventing the onset of insanity.

Thanks to all the members of my research group past and present who have made life so interesting and of course enjoyable at Aston University. Special thanks to Fiona Lydon for lessons in etiquette and disposing of large amounts of cash and to Karen French for assisting Fiona and their friendship. I would like to express my appreciation to Dr Steve Holding at RAPRA technology for running and helping to interpret the gel permeation chromatograms of the polymers synthesised in this thesis. Cheers to Steve Tonge and Val Franklin and especially Karen Dowden (Patch) for proof reading my thesis and advising me in its completion.

Finally I would like to thank Dr. Simon Holland and SmithKline Beechham Pharmaceuticals in addition to the Science and Engineering Research Council for their continued financial support.

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LIST OF ABBREVIATIONS

DMSO	dimethyl sulphoxide
ATR FT-IR	attenuated total reflectance fourrier transformed infra red
DMF	dimethylformamide
DMAc	dimethyl acetamide
SEC	size exclusion chromatography
Mn and Wn	number average molecular weight
DPPA	diphenylphosphorylazide
N-BOC	tertiary butyloxycarbonyl protected amine function
Mw	weight average molecular weight
DCC	dicyclohexylcarbodiimide
rpm	revolutions per minute
DMAP	dimethlyaminopyridine
DMAPtos	dimethylaminopyridine ρ-toluene sulphonate
DIC	di *iso*-propylcarbodiimide
TEA	triethylamine
TEMED	tetramethylethylenediamine
TMP	trimethylphosphate
TBAB	tetrabutyl ammonium bromide
SEM	scanning electron microscopy
HPMCAC	hydroxypropylmethylcelluloseacetate succinate
pH_{min}	pH of minimum solubility
RES	reticular endothelial system
PLG	poly (lactide-co-glycolide)
PVA	polyvinyl alcohol
DCM	dichloromethane
PEO	poly (ethylene oxide)
PPO	poly (propylene oxide)
SUV	small unilamella vesicles
DSPC	distearoylphosphotidylcholine
PEG	poly (ethylene glycol)

DOX	doxorubicin
EPR	enhanced permeability and retention
SMANCS	styrene maleic anhydride neocarzinostatin
HCC	hepato cellular carcinoma
ATP	adenosine triphosphate
υ	wavenumber
asym.	asymmetric
def.	deformation
sym.	symmetric

1. CHAPTER 1

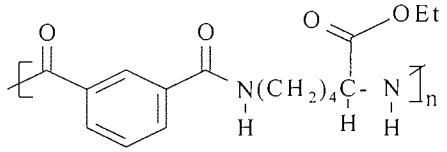
INTERFACIAL SYNTHESIS OF SPECIALTY POLYMERS

1.1 OVERVIEW

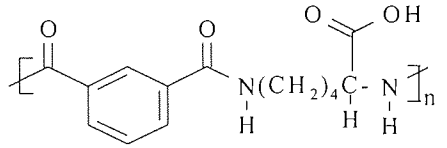
This thesis is concerned with the synthesis and characterisation of a number of functional polyamides. Some of the potential applications of such functional polymers are discussed in Ch. 2. The type of applications envisaged would exploit the pH dependant solubility characteristics of the polymer. Perhaps the most common example of such behaviour in a biomedical application is the use of enteric polymers to coat orally administered pharmaceuticals. This application is discussed in Sec. 2.1. Of particular interest was the possibility of exploiting differences in physiological pH and the pH within solid tumours to effect precipitation of a polymer within the extracellular space of such tumours. This application is discussed in more detail in Sec. 2.5. The desired functionality of the polymer is achieved by incorporating weakly charged hydrophilic carboxyl groups along the backbone of the polymer balanced by a degree of hydrophobicity within or pendant to the backbone. The solubility characteristics of such a polymer could potentially be modified by structural modification through variation of the starting monomers or copolymerisation of a variety of monomers. In view of the possible biomedical applications of such polymers, biodegradability was perceived to be an important feature of the polymers. A synthetic strategy was followed whereby polyfunctional precursors, including naturally occurring metabolites could be condensed with hydrophobic diacid chlorides using a low temperature polymerisation technique that avoided involved protection/deprotection reactions and monomer purification. Successfully synthesised polymers are listed in Fig.1.1. Interfacial polycondensation was particularly attractive since the rapidity of the main polymer forming reaction meant that potential crosslinking reactions through certain side chain functionalities can be outpaced thus avoiding the type of side chain functional group protection required in peptide syntheses for example. Effort was concentrated on the natural diamino acids, L-lysine and L-ornithine although shorter diamino acids such as L-diamino propionic, DL-diamino butyric and *meso*-diamino succinic acid were also investigated with less success.

Unless specified, all amino acids referred to in the subsequent text were naturally occurring L-isomers. This thesis will concentrate on the synthesis and characterisation of successfully synthesised polymers although potential reasons for the failure of some attempted polymerisation reactions are discussed in Ch. 7.

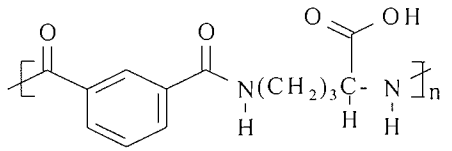
Fig. 1.1 Examples of target polymer structures.



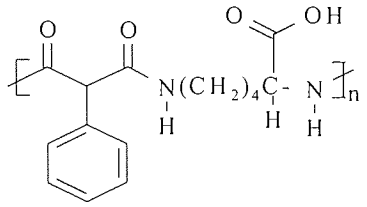
Poly (lysine ethyl ester *iso*-phthalamide)



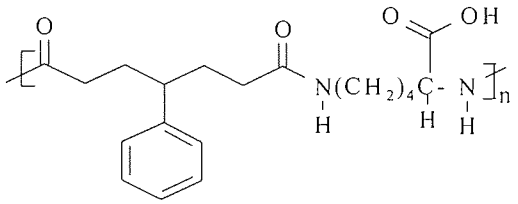
Poly (lysine *iso*-phthalamide)



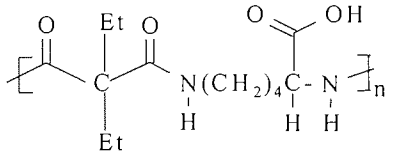
Poly (ornithine isophthalamide)



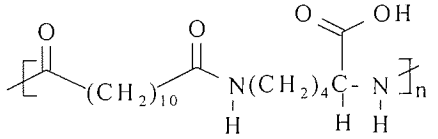
Poly (lysine phenyl malonamide)



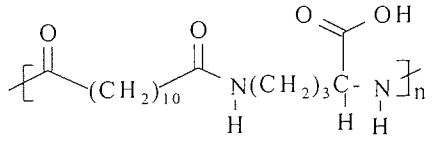
Poly (lysine phenyl glutamide)



Poly (lysine diethyl malonamide)



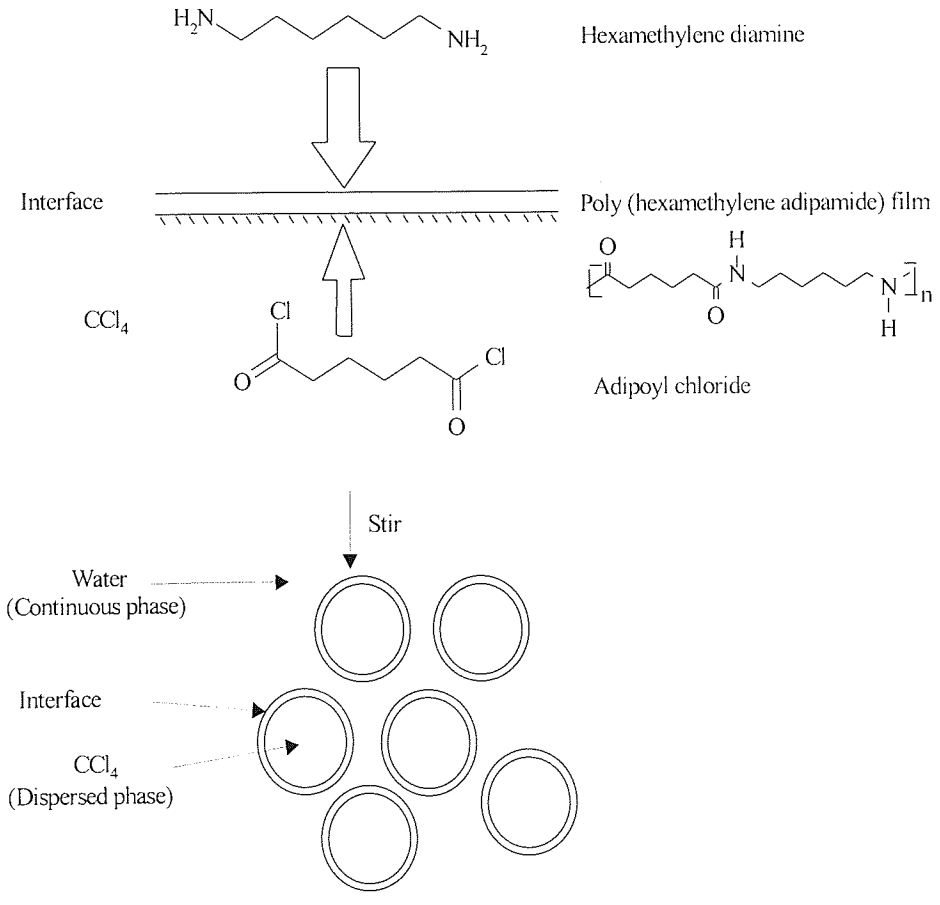
Poly (lysine dodecanamide)



Poly (ornithine dodecanamide)

In its simplest form an interfacial reaction takes place at or adjacent to the interface of two immiscible liquids, typically an organic solvent and water, where the organic phase contains a diacid halide or similarly reactive species and the aqueous phase contains a compound with an active hydrogen in the functional group such as a diamine, bis-phenol or dithiol and usually an acid halide acceptor[1]. Aliphatic diols are not normally sufficiently reactive to participate in interfacial polycondensations. Under certain circumstances their reactivity can be enhanced sufficiently to allow them to be considered[2-4]. The two phases provide a controlled delivery of reactive species to the site of reaction through diffusion[5]. The rate of reaction can be increased by mixing the two phases. The interfacial area and thus contact between reactive species is considerably enhanced and the distance the reactive species must diffuse is reduced (Fig. 1.2).

Fig. 1.2 Reaction of a diamine and a diacid chloride at the interface between two immiscible liquids.



FT-IR spectroscopy was used to determine whether condensation between the diacid chloride and diamine had taken place to produce the amide linkages within the polymer backbone. The technique was also able to indicate the presence of anhydride linkages which formed under certain reaction conditions (Sec. 3.7 and Sec. 4.5.2.3).

An extensive investigation into the polymerisation of diamino acids and their esterified derivatives, using both interfacial polycondensation (Ch. 4) and a novel miscible mixed solvent technique (Ch. 3), was undertaken to determine the effect of reaction parameters on the molecular weight and yield of the polymers and on the reproducibility of the techniques. Conditions were identified for both types of polycondensation which allowed the reproducible synthesis of acceptably high molecular weight functional polyamides. The molecular weight averages were determined by gel permeation chromatography. The technique was particularly useful for analysis of the polyamide samples bearing esterified carboxylic acid functional groups although proved to be rather problematic for the analysis of polymers bearing free carboxylic acid groups. The results are presented in Ch. 5 along with some discussion of the experimental peculiarities observed with the free carboxyl bearing polymers.

Potentiometric titrations were used to investigate possible secondary structure formation at low degrees of ionisation. Van der Waals interactions between the hydrophobic moieties can result in the formation of amphiphilic hypercoiled structures at low degrees of ionisation (Fig. 6.5 and Fig. 6.6). A more detailed discussion of the mechanism and consequences of this phenomenon on the potentiometric titrations of the polyamides is given in Sec. 6.3.

1.2 INTERFACIAL SYNTHESIS.

1.2.1 Introduction

The interfacial synthesis of high molecular weight polymers was first reported in the 1940's[1,5]. The technique received considerable attention during the 1970s and has been applied to the successful synthesis of a variety of polymers including polyesters, polyamides, polyurethanes, polycarbonates, polyiminocarbonates, as well as a wide range of copolymers involving mixed linkages in the backbone[1,5-7]. Although large scale synthesis is limited mainly to polycarbonate production[5] the technique is widely used in the lab-scale batch synthesis of speciality polymers that are difficult, if not impossible, to obtain by other means.

Interfacial polymerisation is a low temperature, non equilibrium process occurring at or near the interface of two immiscible liquids such as carbon tetrachloride and water[5,8]. It offers several advantages over the high temperature melt polymerisation process. These include much higher rates of reaction and greater tolerance to reagent impurities and stoichiometric ratio[5,8]. Also, since the reaction proceeds at room temperature, heat sensitive[9,10] and infusible[11-15] polymers can be synthesised. Interfacial polycondensations can be conducted in a laboratory blender, whereas, specialised equipment, resistant to high temperature and pressure, is required for the melt process. The interfacial synthetic process is extremely complex involving many interrelated variables that will be discussed in more detail further in Sec. 1.4. These parameters often make process scaling-up difficult and this may partially explain why the process has not been adopted for the large scale production of condensation polymers. Other factors include the higher cost of starting materials and processing difficulties that arise because of the finely divided form of the final polymer[5]. These factors may offset the financial savings incurred by the heating and equipment costs involved in the melt polymerisation of conventional polyamides. If, however, the melt process is unsuitable for the synthesis of certain polymers, such as functional polyamides of the type proposed in this thesis, then the interfacial process may offer an alternative.

Despite the apparent complexity of the interfacial reaction it is an extremely useful synthetic method for the polymer chemist since it enables polymers with adequate molecular weights to be produced under a variety of conditions and it is this synthetic versatility that makes interfacial synthesis an attractive option.

1.3 INTERFACIAL SYNTHESIS AS A ROUTE TO HIGH MOLECULAR WEIGHT POLYMERS

The subsequent discussion of factors effecting interfacial polycondensations will be limited to the synthesis of polyesters and polyamides although many of the arguments apply to other polycondensations. Interfacial reactions proceed at relatively high rates (k typically $>10^6$ $Lmoles^{-1}s^{-1}$) and are essentially irreversible at room temperature giving high yields often within a few minutes.

The extremely high rates of reaction involved indicate that the observed rate of reaction is generally diffusion controlled, i.e. the rate limiting factor is not the chemical reactivity of the monomers but the rate of diffusion of the monomers to the reactive site[1,5]. Non-equivalence in monomers can, to some degree, be tolerated as long as there is an equivalence of reactive species at the site of reaction. Factors that effect the concentrations of the reactive species at the locus of polymerisation, therefore, play an important role in the overall yield and final molecular weight distribution of the product[8]. Such factors are discussed in greater detail in section 1.4.

1.4 MECHANISM OF INTERFACIAL POLYAMIDATION

Interfacial syntheses of polyamides from diacid chlorides and diamines proceeds via the Schotten-Baumann nucleophilic substitution reaction[1,5,8]. A protonated amide is formed, via an intermediate complex, that rapidly loses a proton to an amine group. It is usual to add an acid acceptor to the aqueous phase where the free amine is regenerated, since protonated amines are not reactive. An excess diamine or a variety of organic and inorganic bases can be used.

It is generally accepted that the site of reaction is in the organic phase adjacent to the interface and that the main function of the aqueous phase is to serve as solvent for both the diamine and acid acceptor and to remove the acid generated during the reaction[1], although this may not be true if hydrophilic diamines are used[15]. During the initial stages of the polycondensation the diamine, which usually has an appreciable partition potential for the organic solvent, enters the organic phase and is acylated to form acid chloride terminated oligomers. Subsequent diamine then reacts with the oligomers increasing their chain length. High molecular weight material forms as a result of the new diamine reacting with acid chloride terminated oligomers rather than fresh diacid chloride entering the polymerisation zone. There is some diffusion of oligomers away from the polymerisation zone but diffusion of the polymeric chains becomes slower with an increase in size. Polymer with the highest molecular weight is obtained when the rate of transfer of reactive species to the polymerisation zone provides an equivalence of reagents whilst the chains are dissolved. No significant increase in molecular weight is seen following precipitation of the polymer.

1.5 FACTORS EFFECTING INTERFACIAL SYNTHESIS

The reaction variables that play an important part in an interfacial polycondensation are reviewed along with their relevance to the types of reaction systems investigated. Many people are familiar with the "Nylon rope trick"[16] where a continuous fibre of polymer is pulled from the interface between two immiscible phases. The two liquids are typically carbon tetrachloride containing adipoyl chloride against an aqueous solution of hexamethylene diamine and sodium hydroxide. At the interface of the two liquids an insoluble film of poly (hexamethylene adipamide) forms rapidly. If the film is removed from the interface the unreacted monomer in the two phases come into contact with each other and react to form fresh film. The reaction is so rapid that film can be continuously removed as a coherent collapsed tube until one of the reagents is exhausted. If, however, the film is not withdrawn the reaction rapidly comes to a halt since the rate of diffusion of the reagents across the interface is reduced by the film. The reaction slows and eventually stops as the thickness of the film increases.

If the polymer film is examined it can be seen that the surface facing the aqueous phase is smooth whereas the side facing the organic solvent is pitted and appears to be rougher. A decrease in molecular weight from the aqueous face to the organic is predicted by Morgan[8]. The gradation was attributed to reduced mobility of polymer and intermediates within the solvent swollen precipitated polymeric matrix. This drastically reduces the rate of polymerisation after precipitation.

The degree of swelling of the precipitated polymer by the organic solvent effects the final molecular weight, since this will determine the mobility of lower molecular weight oligomers and monomers within the matrix. Diffusion of diamine through the film results in a secondary growth of low molecular weight polymer on the organic face of the film. An ideal system would allow high molecular weight to be achieved before precipitation to a compact film preventing the formation of low polymer by the process mentioned above. The heterogeneity of membranes produced at the interface of an oil/water system is supported by Janssen and Nijenhuis[17,18]. Oil droplets were encapsulated by a thin membrane produced by the polycondensation of *tera*-phthaloyl chloride with diethylenetriamine at the interface of an oil-in-water dispersion. This was followed by the growth of a porous sublayer on the side of the membrane adjacent to the oil droplet. A model for wall growth is proposed whereby the initial thin film is penetrated and swollen by the aqueous phase whilst diamine diffuses into the oil phase to react with the *tera*-phthaloyl chloride. As the diamine reacts with the *tera*-phthaloyl chloride within the droplet the volume of the oil phase is decreased and droplets of the aqueous phase appear on the internal face of the capsules and these coalesce to form larger droplets. The coalescence is stopped by precipitation of the polymer which occurs at a critical molecular weight. As the membrane thickens the rate of diffusion of the diamine and hence the rate of polymerisation is reduced and larger droplets form before precipitation of the polymer. The increase in the size of the pores is cited as evidence for the reduced diffusion of the diamine.

Chern *et. al.*[12] used Attenuated Total Reflectance Fourier Transform Infra-Red Spectroscopy (ATR FT-IR) to examine membranes made by the unstirred polycondensation of 1,2,4,5-benzenetetraacyl chloride with various diamines. The results showed differences in the chemical structure between the surface of the membrane facing the aqueous phase and that facing the organic phase. If no acid acceptor was used the side adjacent to the aqueous phase showed a stronger characteristic amide absorption (1540, 1640cm^{-1}) than that of the organic phase and the latter showed characteristic acid absorptions (1720 cm^{-1}). Bubbles of gas, assumed to be hydrogen chloride, were produced on the film facing the organic phase. Similar results are seen if sodium carbonate is present. During the initial stages of the reaction no difference in the two faces is apparent. The results are interpreted in terms of a diffusion mechanism whereby diamine transfers to the organic phase and reacts with the acid chloride to form a thin polymeric film. As a result, diffusion of the diamine through the film and removal of hydrogen chloride to the aqueous phase is slowed which allows a build-up of hydrogen chloride in the organic phase, adjacent to the film, thereby promoting the hydrolysis of the diacid chloride. The similarity of the two sides during the initial stages of the reaction indicate that the film is growing towards the organic phase and that the site of reaction is located in the organic phase. If tertiary butyl ammonium chloride is added it is found that the rate of amidation is increased due to an increase in the transfer of the diamine to the organic phase.

1.5.1 Stirring in interfacial polycondensation

Non stirred interfacial syntheses are generally application driven, for example the synthesis of thin films or membranes such as the reverse osmotic membranes proposed by Cadotte *et. al.*[19]. Whitfield *et. al.*[20] developed a novel process for the shrink protection of textile fibres by an interfacial method. The fibres where firstly immersed in a diamine solution, followed by a diacid chloride solution, resulting in the formation of a protective coating of polymer between 10 and 20 NM thick. Improved resistance to abrasion, wrinkling and chemical attack were also observed.

As noted previously, in order to obtain high molecular weight material with the narrowest molecular weight distribution, the precipitated film must be continually removed from the interface in order to reform fresh interface. This requires the film to possess sufficient strength to allow removal without breakage. In a stirred interfacial reaction this is not the case and the final polymer may be precipitated or dissolved in either of the phases[21].

High speed stirring is usually employed in an interfacial reaction in order to increase the interfacial area and reduce reaction times[1,5,21,22]. Stirring results in shear forces within the liquid which result in turbulent flow in addition to mass flow of the liquid. If two immiscible liquids are present the resultant shear forces generated between the phases lead to the formation of a dispersion of droplets of one phase, usually the lighter phase, within a continuous phase, normally the heavier phase. Phase inversion may occur at any stage if the dispersion becomes unstable. This may result from the conversion of the monomers or precipitation of the polymer. As the intensity or efficiency of the stirring is increased droplet size will be reduced creating a finer dispersion. As the droplet size is reduced the distance that monomer within the droplet must travel is reduced and the observed rate of reaction increases, approaching the limiting chemical reaction rate. Fluid motion within the continuous phase enhances the delivery of reactive species and the removal of product at the interface enhancing diffusion rates by forced convection and turbulence[5]. When the resulting polymer is insoluble in either phase then vigorous stirring can tear apart the encapsulated droplets. Fresh interface is rapidly created minimising the lowering and broadening of the molecular weight distribution noted in the case of nonstirred systems. The dispersion is in dynamic equilibrium, with the droplets continually coalescing and reforming, distributing the reactive species and precipitated or dissolved polymer throughout the bulk. Heat generated in the reaction is more efficiently dissipated by efficient stirring.

The design of any reactor is important and should take into account the stirring requirements of the reaction. The rate of reaction in an interfacial reaction is usually diffusion controlled and processes that enhance the diffusion of reactive species to the interface should improve the rate of reaction. Increased turbulence creates more interfacial area thus enhancing the observed rate of reaction by increasing the contact between reactive species and reducing the distance for diffusion. Mass flow of the system results in large scale movement of liquid, including the turbulent eddy currents, to give a more homogeneous system. Large scale mass flow maintains the overall bulk homogeneity of the dispersion, whereas turbulence induced eddy currents provide small scale mixing. Vortexing within a stirred system is undesirable since it induces lateral flow of material under centrifugal force away from the impeller. Mixing efficiency is at a minimum since laminar circular flow results in reduced shear within the liquid. The ratio of impeller size to reactor diameter, position of impeller and design of reactor all play an important role. Disruption of swirling can be achieved by the inclusion of baffles in the reactor or by eccentric positioning of the impeller, both of which can induce more favourable axial and lateral flow patterns.

Choice of impeller type effects the flow pattern set up in the system. A marine type propeller induces initial axial flow and when positioned near the interface of the two liquids will result in the lighter phase being drawn into the heavier phase. A flat paddle or turbine design will result in initial radial motion of the liquid. Both forms of impeller give good axial and radial flow in an adequately baffled system by the interaction of the streams with the vessel walls. Varying the type of impeller will alter the ratio of mass flow to turbulence, for example an homogeniser will provide a high turbulence to flow ratio whereas a flat paddle will induce a lower degree of turbulence for the same power input in an otherwise similar system. High turbulence creates a greater interfacial area within a system and so mixing devices that generate a high turbulence to flow ratio are often employed in interfacial reactions. Common types of reactor include Waring blenders and homogenisers as well as more simple overhead stirrers used with baffled resin flasks.

Often the effect of stirring, as an experimental variable, is removed by unnecessarily high rates of stirring. This may ensure that small experimental variations in fluid motion will have a minimal effect on the outcome of a reaction. Too much stirring can be detrimental to a reaction as the droplet size in a dispersion, though initially reduced with increased stirring, eventually increases in size. The power used in the impeller is eventually dissipated as heat within a reaction and this will alter relative rates of polymer forming reactions and side reactions. Heat generated during the reaction can result in evaporation of volatile organic solvent altering the phase volume ratio and molarity. The concentration of the acid chloride increases whilst the interfacial area is reduced. Rates of reaction may be increased to such an extent that the initial precipitation of high molecular weight material generated on formation of the dispersion forms a gel and this completely disrupts stirring. Lowering and broadening of the molecular weight distribution may occur for the reasons explained previously unless the initial dispersion was so fine as to result in the exhaustion of the material within the droplets during the initial film formation. Surfactants can reduce the droplet size whilst maintaining the fluidity of the reaction although they may prove difficult to remove during purification.

1.5.2 Chemical reaction rate

Assuming that the most efficient mixing possible is achieved then the observed rate of reaction will depend on the chemical reaction rate of the species involved. This can be as high as 10^{6}Lmole^{-1}s^{-1} for the reaction between an aliphatic diacid chloride and an aliphatic diamine and may be as low as 10^{-2}Lmole^{-1}s^{-1} for the reaction between an aromatic bis-chloroformate and an aromatic diamine[21]. The main requirement for a successful polycondensation is that the main polymer forming reaction should proceed faster than any relevant side reactions[1,5,23].

1.5.3 Side reactions in interfacial synthesis

Several important side reactions may occur in an interfacial polycondensation reaction. These may simply reduce the molecular weight of the polymer by consumption of one of the monomers or may cause structural modification to the polymer backbone altering both physical and chemical properties.

1.5.3.1 Hydrolysis of the diacid chloride

In most cases the most important side reaction is the hydrolysis of the diacid chloride. Morgan[1] states that the hydrolysis of acid chlorides takes place exclusively in the aqueous phase and rates of acid chloride hydrolysis under various experimental conditions have been studied[24]. Hydrolysis is particularly troublesome when using the lower molecular weight aliphatic acid chlorides, with less than seven methylene units, when the limiting factor in their hydrolysis is considered to be their extraction into the aqueous phase. These short chain diacid chlorides are inherently more reactive than aromatic diacid chlorides, but are also readily extracted into the aqueous phase. Longer aliphatic acid chlorides such as sebacoyl chloride and less reactive aromatic acid chlorides such as *tera*-phthaloyl chloride generally give better results in both terms of yield and molecular weight distribution[8,25]. Hydrolysis of acid chloride terminated oligomers reduces the molecular weight of hydrolytically sensitive polymers. Saotome *et. al.*[25] ascribe the low reduced viscosities of the polyamide synthesised from adipoyl chloride and lysine to the formation of inactive carboxyl end groups by hydrolysis of acyl chloride ended oligomers. The site of reaction is taken to be partially or wholly in the aqueous phase in this case since the polymers remain dissolved in the aqueous phase at the end of the reaction because of the pendant carboxyl groups on the lysine moiety. Wang *et. al.*[23] found that polyesters synthesised from bis-phenols with pendant carboxyl groups had much higher intrinsic viscosities when the ratio of bis-phenol to diacid chloride was 0.9 rather than 1.2. This is explained in terms of the structure of the oligomers formed at the initial stages of the reaction.

In the first case the oligomers with pendant carboxyl groups will have acid chloride groups at both ends and their solubility will be reduced in both phases restricting them to the interface. Increasing the amount of bis-phenol results in a greater proportion of phenoxide ended oligomers and a reaction which must occur wholly on the aqueous side of the interface.

1.5.3.2 Formation of low molecular weight material.

Linear condensation polymers produced by the melt process generally have a normal statistical molecular weight distribution because the equilibrium nature of the reaction means that a continual interchain exchange between amide groups occurs[1]. Low temperature solution methods employ reactive species in reactions that are essentially irreversible at room temperature. The polydispersisities of condensation polymers produced by interfacial synthesis depend on the nature of the polymerisation system. If the polymer remains in solution in the organic phase then a normal statistical distribution approaching that seen in a melt polymerisation is seen. If the polymer precipitates rapidly then increasingly broader distributions are observed.

Many of the lower molecular weight oligomers are cyclic, the formation of which is reviewed by Zahn and Gleitsmann[26]. In a further review of the literature Morgan[1] concludes that in addition to factors such as reaction rate and steric factors, solvent sensitivity of polymer and oligomers and increased dilution of the system will increase the amount of cyclic oligomers. These conclusions are supported by the work of Cleaver and Pratt[27] who observed a decrease in occurrence of cyclic oligomers from 14.1% (with 81.3% linear polymer) to an almost quantitative production of linear polymer in an interfacial reaction between hexamethylene diamine and 2,2'-tetramethylene-bis-[4,4-dimethyl-5(4H)-oxalone] in pyridine by increasing the reagent concentrations from 0.1M to 1M. The inherent viscosity increased from 0.15 dLg^{-1} (averaged for the two fractions) to 0.56 dLg^{-1} (in m-cresol at 0.3%).

The increased polydispersisities noted in polymers formed by the interfacial process have led to the process being abandoned in favour of the melt process. In the synthesis of speciality polymers this may not be an option. Removal of oligomers by selective fractionation in the latter case is possible although this will reduce the ultimate yield increasing overall cost of synthesis.

1.5.3.3 Imide formation.

Imide formation resulting from the reaction of acid chloride with secondary amides within the backbone can be problematic leading to branching of the polymer, particularly with high concentrations of diacid chlorides. Morgan[28] found that the branches in interfacially synthesised 6-10 polyamide, could be cleaved by treatment in sulphuric acid or by melt treatment. Prolonged melting resulted in interchange of chain terminating amine and carboxyl groups with amide groups within the backbone. A molecular weight distribution closer to the normal statistical distribution seen with melt polymerisations results with a lower overall average. Imide formation may the preferential reaction with some sterically restricted diacid chlorides such as phthaloyl dichloride[28] and an unusual example of imide formation under specific reaction conditions is discussed in Sec. 1.8. Secondary diamines such as piperazine that cannot form imide structures have produced polyamides in good yield.

Concentration ratio and phase ratio effects.

For optimum results in an interfacial polymerisation, a balance of monomers coming into contact in the polymerisation zone must be achieved. Morgan[21] found that similar trends in response to changes in stirring, concentration and phase ratio on the final yield and molecular weight distribution of a polymer, could be attributed to the solvent sensitivity of the polymer. Three groups were identified

In the first case, rapid precipitation of the polymer in a solvent swollen state occurs. Attainment of the maximum possible molecular weight is dependent on rapid stirring for the reasons discussed previously.

Since high stirring rates effectively increase diamine availability, to achieve the required balance in reagents, a lower concentration of diamine or a more concentrated organic phase is required. Coincidence of peak molecular weight with minimum polydispersity and maximum yield were noted by several workers[1].

In the second case, rapid precipitation to a non solvent swollen state occurs. Polymer and oligomeric mobility is practically nil and no increase in molecular weight occurs after precipitation. These reactions seem to be relatively insensitive to many operation variables, with maximum molecular weight being attained in dilute systems where the polymerisation zone approaches that of a monolayer at the interface. These conditions necessitate hydrolytic stability or low water solubility of the diacid chloride.

Finally the polymer may remain dissolved in the organic solvent. Comparison of the polymerisation of piperazine with sebacoyl chloride in carbon tetrachloride (a nonsolvent for the polymer) and in dichloromethane (a solvent for the polymer) show an increase in the inherent viscosity from 1.5 dLg^{-1} to 3.1 dLg^{-1} (*m*-cresol, 25°C) when the polymer remained dissolved. Both maxima occurred at similar concentrations of acid chloride (~1.0M) and diamine (~0.1M). Changing the organic solvent to chloroform, a better solvent for the polymer, had a detrimental effect on the inherent viscosity. This seemingly unusual result was attributed to the increased viscosity of the resulting solution of polymer in chloroform at lower concentrations of polymer.

In none of these cases do the authors consider the possibility that the resulting polymer may be dissolved by aqueous phase. This would be possible if the diamine contained additional hydrophilic functionalities. In such cases, the site of reaction may also differ from that widely accepted as the organic side of the interface.

In all cases reported rapid addition of the organic phase to the aqueous phase gave the best results. Slow addition of either phase results in an initial excess of one reagent.

1.5.5 Stoichiometric ratio

Although an exact stoichiometric ratio is not essential for successful interfacial polycondensation, large deviations generally have an undesirable effect on overall yield and molecular weight in stirred systems. Unlike in unstirred systems where the polymerisation is disrupted by the precipitation of a polymeric film, in a stirred system the polymerisation continues until one of the reagents is exhausted, so the relative excess of one reactant will increase dramatically towards the completion of the reaction. Some systems are less sensitive to stoichiometric imbalance because of the overriding effects of acid chloride hydrolysis or polymer precipitation. Excess of either reagent means that the concentration or volume of one of the phases and hence the phase ratio must change and the previous arguments will still apply.

Yield and molecular weight may be improved by use of a slight excess of one reagent under the following conditions. Dilution of the aqueous phase can lead to an unfavourable shift in the equilibrium partition coefficient of the diamine in favour of water, which may be compensated by a slight excess of diamine It would also compensate for inefficient stirring and could be used as an alternative to increasing the aqueous phase volume.

An excess of diacid chloride can be beneficial where hydrolysis is a problem, but since diacid chloride hydrolysis consumes base then an excess of base would also be required.

1.5.6 Acid acceptor.

Protonation of the amine function by interaction with by-product acid results in non reactive species. The free amine can be regenerated in the aqueous phase by the action of strong base. In the absence of strong base excess diamine can be used as the acid acceptor. Several acid acceptors[21,22] have been used but optimum results are generally achieved with a 2:1 ratio of potassium or sodium hydroxide to diacid chloride or an excess of sodium or potassium carbonate. Excess hydroxide generally reduces yield and average molecular weigh due to increased hydrolysis of the diacid chloride and acid chloride terminated oligomers. Diacid chloride hydrolysis can be further reduced by using dispersions of insoluble oxides of magnesium or calcium[1], which do not increase the basicity of the aqueous phase as much as soluble salts. Organic tertiary amines are generally poor acceptors and the use of pyridine can often be detrimental.

1.6 INTERFACIAL SYNTHESIS OF POLYARYLATES

Polyesters can be synthesised interfacially by the reaction of diacid chlorides with bis-phenoxides[5,23,29-32]. Bis-phenoxides are more reactive than the corresponding bis-phenol and are generally prepared by the addition of strong base, typically sodium hydroxide, to an aqueous solution of the bis-phenol. The resulting bis-phenoxide has much lower equilibrium partition coefficient than a diamine would have in the non polar organic solvents normally used and there is evidence pointing to either phase as the locus of polymerisation.[8,23,27]. The lower rates of reaction between phenoxide and acid chloride have led some workers[33] to propose an intermediate reaction mechanism where the reaction still takes place at the interface, but is no longer diffusion controlled. In this case the overall rate of reaction is defined by the chemical reaction rate. This may be true in some cases, but it is clear that other reactions are still diffusion controlled and stirring is an important experimental variable. Choice of organic solvent in a polyesterification is more important than in polyamidations because of the poor solubility of the bis-phenoxide[23,29]. The solvent must be a solvent or a near solvent for the polymer in order to maintain mobility of oligomers at the interface and allow approach of the diacid chloride to the interface.

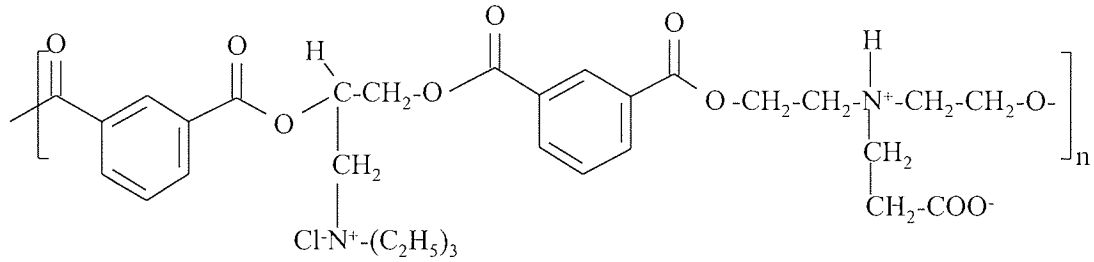
1.6.1 Accelerators in polyesterifications

Accelerators are generally added to an interfacial polymerisation in order to increase rates of reaction and consequently yield and molecular weight of product[1,5,23,30]. There are different types of accelerator and their mode of action differs slightly, but the overall effect is to increase the availability of the bis-phenoxide in the organic phase. This phase transfer effect is common in many organic reactions involving more than one phase, whether it be two liquids or a liquid dispersion of a solid[34]. The phenomenon was first exploited by Shnell[35] in the mid 1940s but only saw significant use in the interfacial process in the 1970s when it was extensively used in the synthesis of polycarbonates and polyarylates. The basic principle is that an organic base or its salt is added to the aqueous phase which then forms a complex with the bis-phenoxide that is more soluble in the organic phase than the bis-phenoxide alone[36,37]. Tertiary and quaternary ammonium, as well as quaternary arsonium, phosphonium and sulphonium compounds have been used with varying degrees of success[1]. The effectiveness of the phase transfer catalyst depends on the resulting solubility of the complex formed between the bis-phenoxide and the catalyst. This is dependent on the choice of organic solvent as well as rate of stirring in addition to the characteristics of the catalyst and the bis-phenoxide[35]. More recently crown ethers and polyethyleneglycols[37,38,39] have been successfully used as phase transfer catalysts. These act by forming host/guest complexes with the gegen ion of the bis-phenoxide, thereby shielding the charge and allowing more rapid passage into the organic phase. A variety of substituted crown ethers showing particular solvent preferences are now available. The polyethylene glycols are believed to act in a similar manor, enveloping the cation associated with the bis-phenoxide. The bis-phenoxide ion is left more exposed in both cases and so its reactivity is enhanced[38].

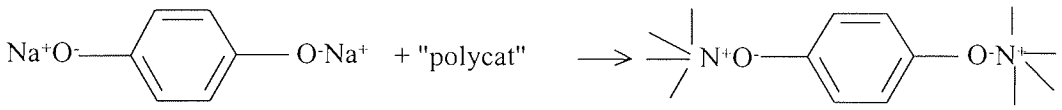
Wang and Nakamura[23] have recently reported using a novel bi-functional polymeric catalyst for the water/oil interfacial preparation of aromatic polyesters with pendant carboxyl functionalities. The catalyst named poly-cat is an amphoteric polymer bearing quaternary ammonium groups pendant to the backbone and tertiary amine groups within the backbone.

This novel catalyst is postulated to exhibit two modes of action (Fig. 1.3). Firstly, the quaternary ammonium groups complex with the bis-phenoxide as with conventional quaternary catalysts, increasing the transfer of the bis-phenoxide to the organic phase. Secondly, the reactivity of the diacid chloride is enhanced by complexing with the amino acid moiety in the main chain. The transfer rate of the acid chloride/poly-cat adduct to the aqueous phase is also increased. The synthesis of this polymeric catalyst is described in Sec. 1.8.

Fig. 1.3 Proposed mechanism for catalytic activity of "poly-cat" in the interfacial polycondensation of bis-phenols and aromatic acyl chlorides.

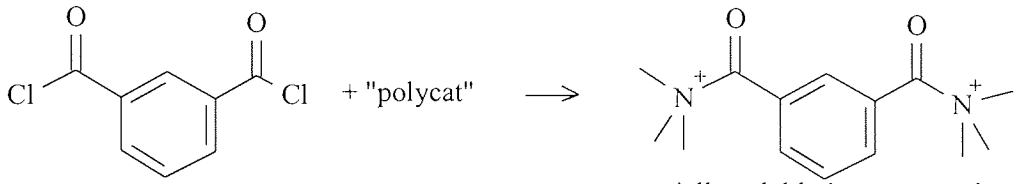


"poly-cat"



sodium bis-phenoxide

partially soluble in organic solvents



iso-phthaloyl chloride

partially soluble in aqueous phase

1.7 INTERFACIAL SYNTHESIS AS A ROUTE TO FUNCTIONALISED OR SPECIALITY POLYMERS

In order to produce a linear polymer with functional groups either within the backbone or pendant to it, the functional group must be non reactive under the reaction conditions involved. In free radical polymerisation, for example, it is relatively simple to produce a polymer such as polyacrylic acid with pendant carboxyl groups. The carboxyl group is not effected by the free radical polymerisation mechanism that forms the backbone of the polymer. The type of linkages in the backbone of the polymers proposed in this thesis result from reactions between functionalities that are also expected to be present as side groups in the final polymer. Side reactions involving these groups must be avoided. Melt polycondensation is not a viable option because of the equilibrium nature of the process. Even if the side groups in the monomer were protected prior to the polymerisation, the reaction conditions would be far too severe to allow a linear polymer to be produced. Hence, monomers that contain ester linkages, for example, could not be melt esterified. A low temperature method is required which essentially means solution polycondensation or interfacial polycondensation. Both have been successfully applied to the synthesis of high molecular weight speciality polymers. The interfacial method is particularly attractive since the restrictions on purity and other factors previously discussed are less stringent than those required for solution polymerisation. In addition, the low temperature solution method requires that the reagents and polymer should dissolve in the organic solvent in order to produce a high molecular weight polymer. In an interfacial synthesis this is not the case and successful polymerisations in which one of the monomers is gaseous[14,15] or insoluble[37] have been reported.

Biodegradability is becoming increasingly important as an environmental issue and also from a biomedical standpoint. Biocompatible materials that undergo controlled biodegradadation to non toxic, metabalizable or excretable end products are extremely desirable for controlled delivery and as temporary mechanical support devices. In addition to these requirements, increasingly sophisticated applications are being found for smart materials that are in some way responsive to in-vivo stimul[40-44].

The smart characteristics of these materials arise as a result of their functionality and the interaction of these functionalities with their environment. The requirements of biodegradability and biocompatibility mean that the existing vinyl and condensation polymers are not ideal candidates for such biomedical applications. New applications are leading to the development of more appropriate polymers specifically designed for particular tasks. Biodegradability within a polymer can be achieved by incorporating hydrolytically or enzymatically cleavable linkages, such as anhydrides, esters, amides or peptides and iminocarbonates within the backbone of the polymer[6,7,43-49]

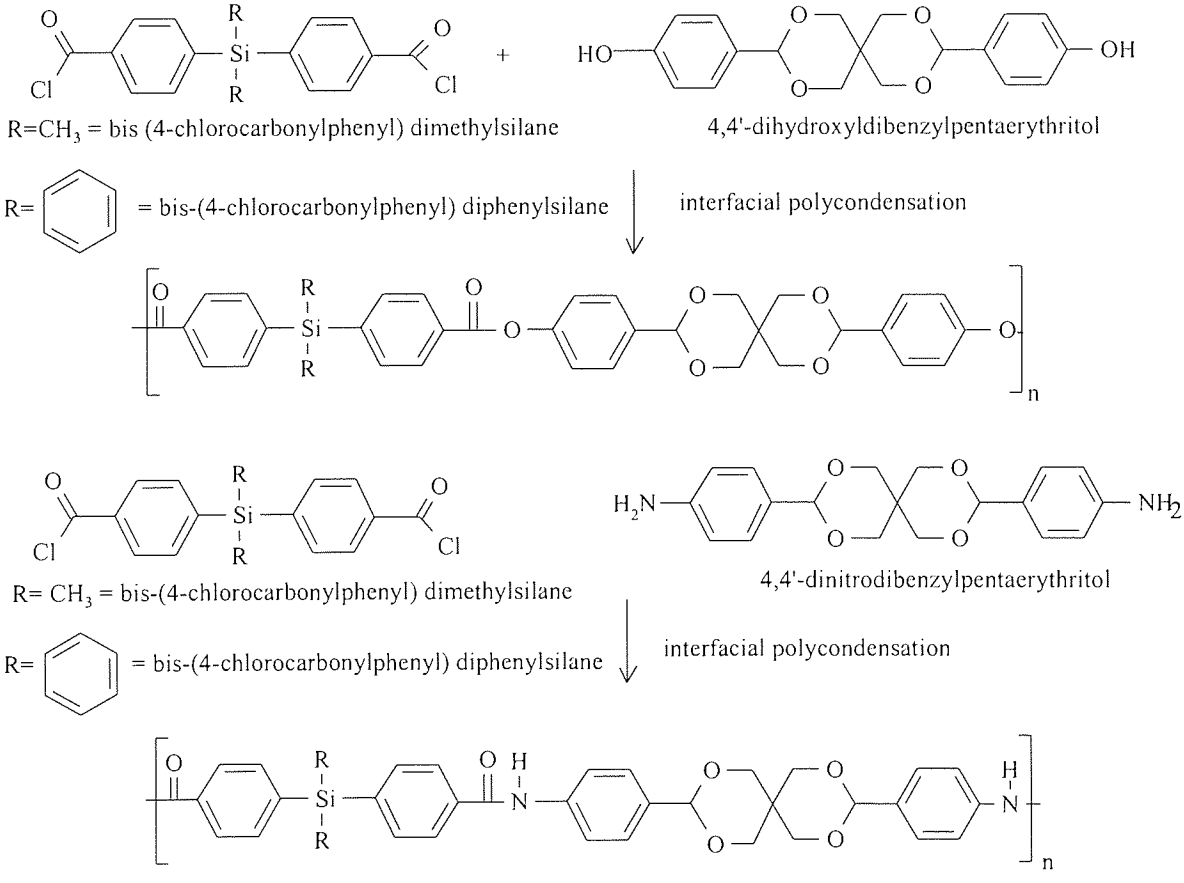
Interfacial synthesis lends itself to the synthesis of novel functional polymers because the high rates of reaction involved allow competing reactions within multifunctional monomers to be outpaced by the chemoselective synthesis of linear polymers possessing pendant functionalities[23]. The nature of the multifunctional monomer determines whether or not protection of one or more of the functionalities is necessary. The rapidity of the main polymer forming reaction between diacid chloride and either bis-phenoxide or diamine means that hydroxyl or carboxyl functions can be tolerated on the monomer dissolved in the aqueous phase. Both polyesters and polyamides containing pendant carboxyl groups have been synthesised[25,23,50]. A tri-amine would need one of the amine functions to be selectively protected even if there were two primary amines and a secondary amine since their activities are similar.[17,18]. Multi-functionality within the diacid chloride usually requires protection of the additional functions to prevent self condensation during synthesis and subsequent reaction[43,44]. The protecting group must be stable during synthesis and the back bone linkages must not be effected during subsequent deprotection or conversion of reactive side groups[51,52].

1.8 SPECIALITY POLYMERS

A number of types of polymers possessing desirable properties have been synthesised by interfacial polymerisation. Kim *et. al.*[53] have synthesised polyesters and polyamides containing spiroacetal units with silphenylene units incorporated into the backbone (Fig. 1.4).

The spiroacetal units impart transparency, hardness, heat and water resistance in conjunction with excellent mechanical properties making them suitable for use as heat resistant materials. In addition, their low bi-refringence makes them useful for photo-memory disks. All these applications depend on the processability of the polymers and so far polymers with spiroacetal moieties attached directly to phenylene units are too rigid resulting in insoluble, infusible and hence non-processable polymers. The inclusion of silphelyne units resulted in polymers that were soluble in polar solvents such as *N,N*-dimethylformamide without loss of thermal stability. The interfacial technique resulted in polyamides with intrinsic viscosities of up to $0.9dLg^{-1}$ (in DMF) with initial decomposition temperatures of 303°C and polyesters with inherent viscosities of up to $0.49dLg^{-1}$ (in DMF) with initial decomposition temperatures of 302°C.

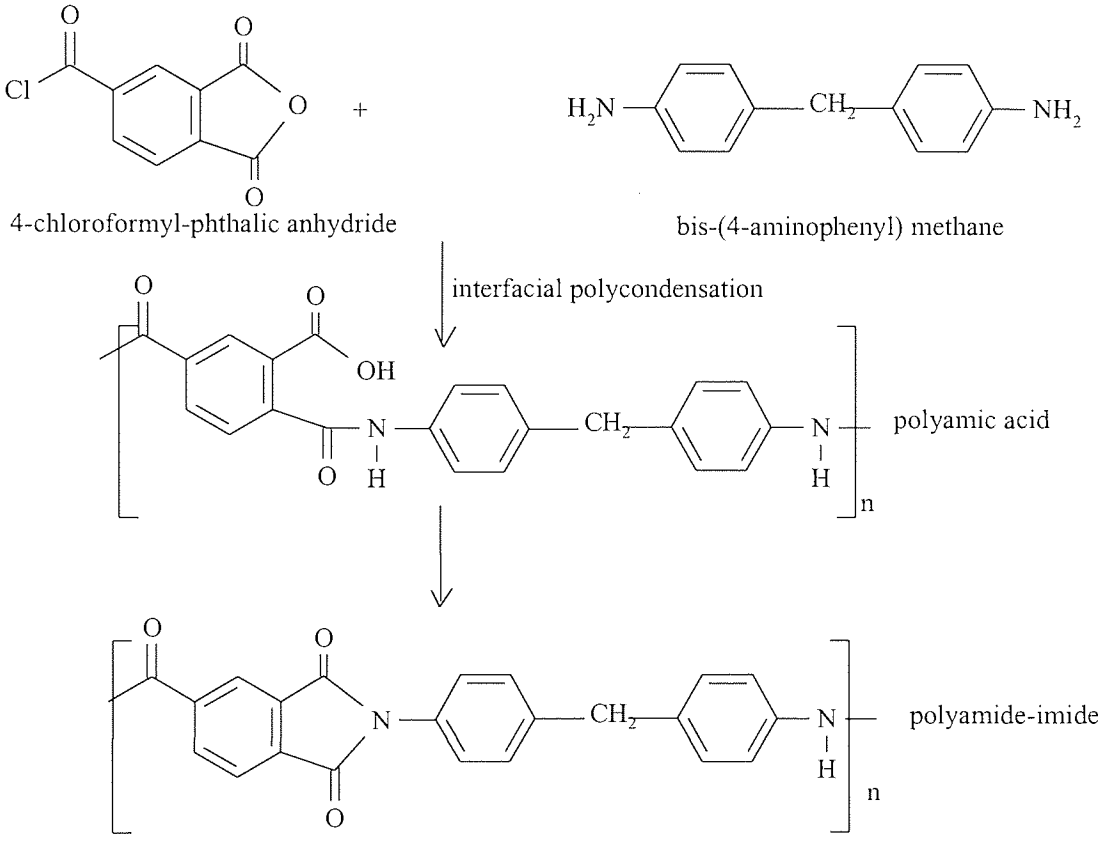
Fig. 1.4 Interfacial synthesis of aromatic polyamides and polyesters containing spiroacetal and silphenylene units.



Another family of thermally stable polymers, the polyimides and the polyamide-imides, have also been prepared by the interfacial technique. Polyamide-imides are normally synthesised by a two step process. A polyamic acid is produced by the solution polycondensation of an aromatic diamine with 4-chloroformyl phthalic anhydride under strictly anhydrous conditions. Subsequent chemical or thermal dehydration gives the polyamide-imides. Imai[11] found that a methylethylketone/water interfacial system gave a product with a higher inherent viscosity of 0.96 dLg^{-1} compared to 0.6 dLg^{-1} when an anhydrous solution method in the same solvent was used (Fig. 1.5). The difference was attributed to the advantageous swelling of the precipitated polymer by water. The mobility of the chains is maintained for a longer period than in anhydrous conditions where precipitation to a non-swollen state occurs within one minute at room temperature. The reaction time is given as ten minutes in the interfacial reaction although no mention is made of the stirring efficiency of the system other than that a Waring type blender was used and that the polymer was precipitated in a swollen state.

Increased stirring efficiency and the use of surfactants to increase the fluidity of the precipitated mass, would be beneficial in reducing the reaction time and hydrolysis of both acyl chloride and anhydride thereby giving a higher molecular weight product. The same polyamide-imide produced by a single phase reaction in dimethylacetamide (DMAc) had an inherent viscosity of 1.06 dLg^{-1}. This is presumably because DMAc is a better solvent for the polymer. The interfacial reaction with DMAc gave a polymer with an inherent viscosity of 0.15 dLg^{-1}. The increased water miscibility of the solvent and increased hydrolysis resulting from the catalysing effect of the solvent[1,2,3,23,54] on the acyl chloride would account for this. Chern *et. al.*[13] attempted the direct interfacial synthesis of polyimides using 1,2,4,5-benzene tetra-acyl chloride with various diamines but found that the reaction products were highly crosslinked.

Fig. 1.5 Interfacial synthesis of polyamic acid from 4-chloroformyl phthalic anhydride and aromatic diamines and their dehydration to polyamide-imides.



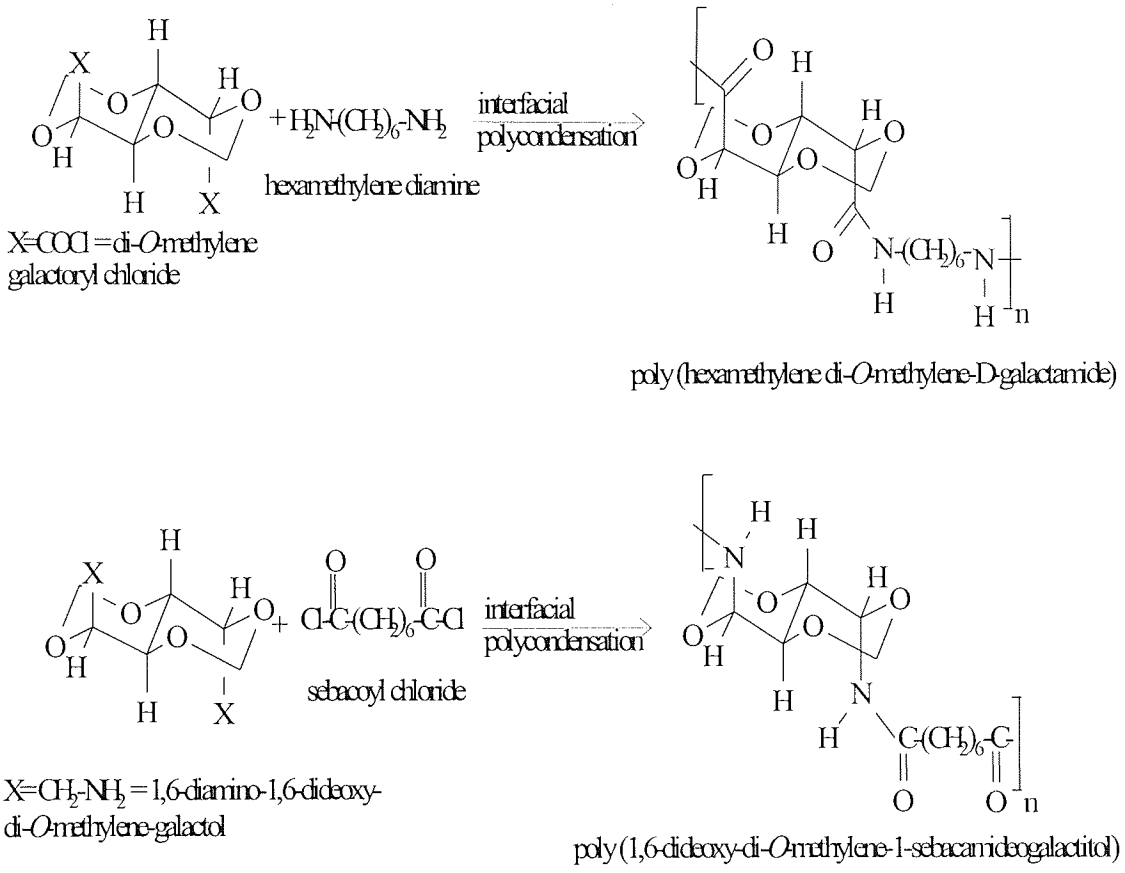
A range of polyimide membranes were subsequently prepared using an unstirred interfacial polymerisation between bis-(methoxycarbonyl) *tera*-phthaloyl chloride in methylene chloride and various diamines in a water/DMAc (10% by vol.) aqueous phase using sodium carbonate as the acid acceptor. The DMAc was necessary because of the poor solubility of the diamines. The polyamic esters thus produced had intrinsic viscosities of 0.27 dLg^{-1} -0.85 dLg^{-1} corresponding to number average molecular weights in the range 18000-55000 (GPC polystyrene standards) with polydispersisities between 1.2 and 1.3. Thermal conversion of the polyamic esters was achieved by heating to 300°C in vacuum.

A major advantage of the interfacial technique was the ability to synthesise extremely thin membranes since the reaction rapidly comes to a halt on precipitation of a film at the interface. Films of less than 0.1μm can easily be achieved. Cadotte *et. al.*[19] used an interfacial technique to prepare composite membranes with an ultrathin polyamide top layer for use as reverse osmosis membranes. A membrane consisting of a polysulphone substrate supporting an intermediate polyethylimine intermediate zone with an ultrathin polyamide top layer were formed by immersing microporous polysulphone membranes in an aqueous solution of polyethylimine, draining off any excess and then covering the support film with a 0.5% solution of *iso*-phthaloyl chloride in hexane. Similar membranes were prepared using piperazine in place of polyethylimine. The polyamide layer provides the selective permeability required, whilst the microporous polysulphone substrate imparted mechanical stability to the film allowing higher throughput of liquid.

In addition to the synthesis of infusible polymers the mildness of the interfacial technique makes it particularly attractive for the synthesis of polymers from thermally unstable monomers. Tsutsumi *et. al.*[55] have recently prepared polyamides containing di-sulphide linkages for use in carbon pastes as positive active material in batteries. Polyamides were produced by a carbon tetrachloride/aqueous NaOH phase process, using diacids and/or diamines containing di-sulphide linkages.

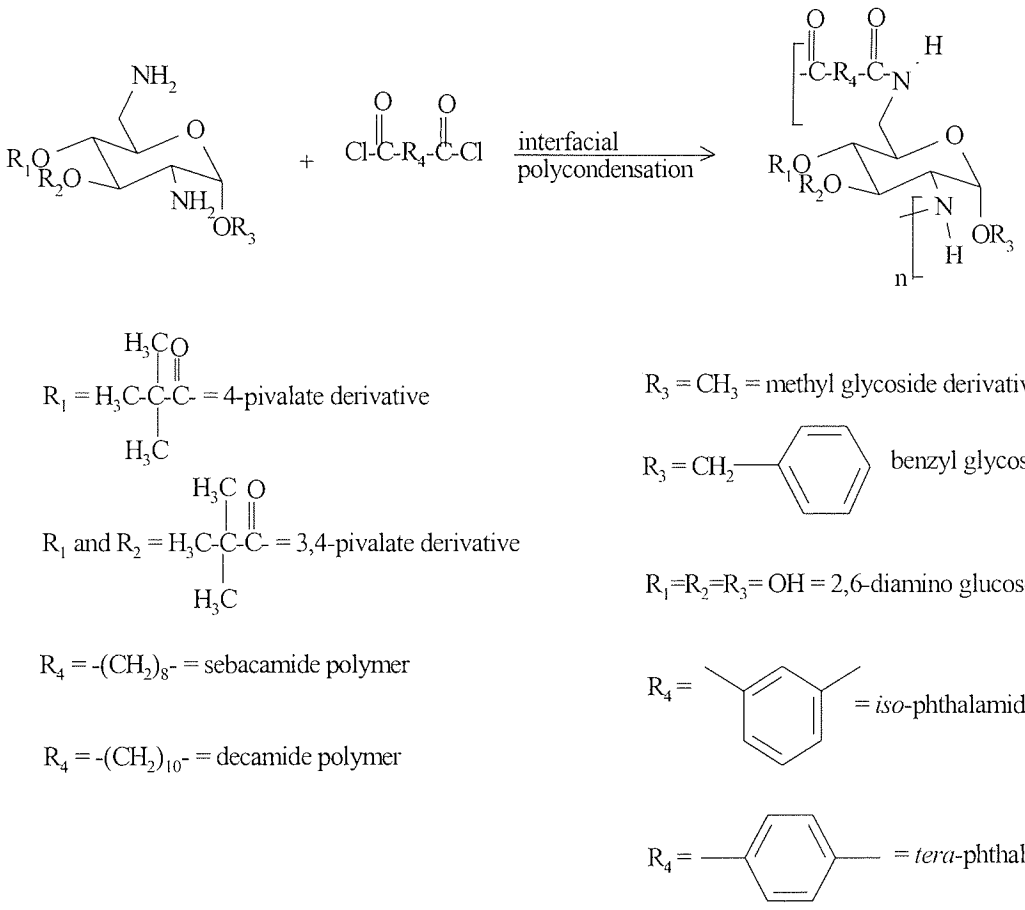
Bird *et. al.*[56] used the interfacial process to synthesise polyamides containing carbohydrate residues after initial attempts at melt polymerisations failed to produce polymers with good fibre forming properties. Polymers with high viscosities approaching that of commercially available Nylon were obtained from derivatised carbohydrates. Carbohydrates converted to diacyl chloride derivatives, such as di-*O*-methylene-D-glucaroyl dichloride, were polymerised with hexamethylene diamine or decamethylene diamine. Alternatively, the carbohydrates were converted to diamine derivatives, such as 1,6-Diamino-1,6-dideoxy-di-*O*-methylene-D-galactitol and polymerised with sebacoyl or adipoyl chloride (Fig. 1.6).

Fig. 1.6 Interfacial polycondensation of carbohydrate derivatives with aliphatic diacyl chlorides and diamines.



Thiem *et. al.*[10] investigated the synthesis of polyamides from renewable resources. A number of differently modified methyl and benzyl 2,6-diaminosacharides were polymerised interfacially with aliphatic and aromatic diacid chlorides using a carbon tetrachloride/aqueous sodium carbonate system. Number average molecular weights in the range 10,300<Mn<24,000 (GPC), compared to values of 2000-3000 for solution polycondensation in dimethylsulphoxide or *N,N*-dimethylacetamide, were obtained with an equivalence of monomers (Fig. 1.7).

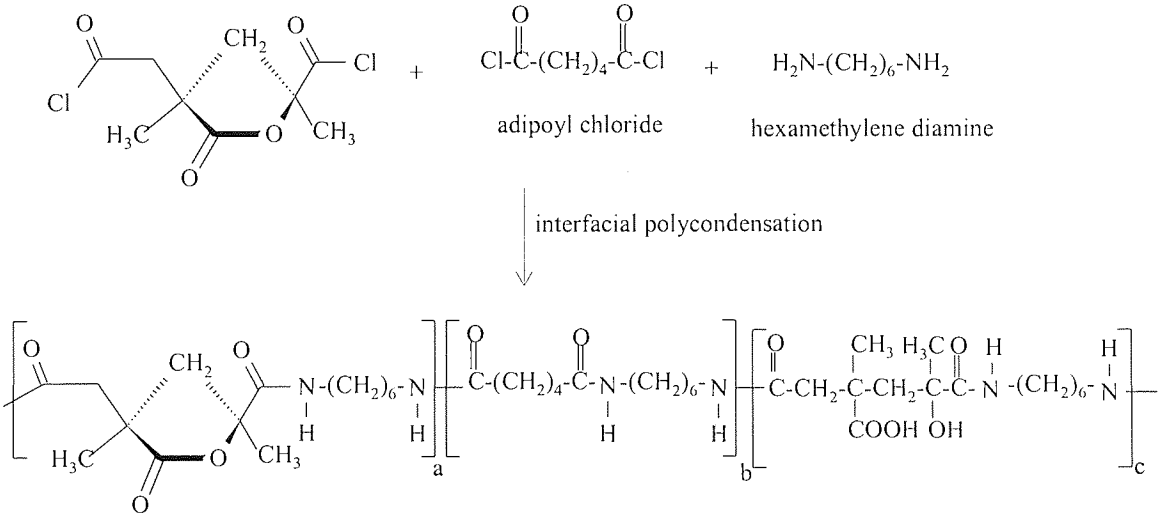
Fig. 1.7 Aliphatic and aromatic polyamides based on 2,6 diaminosaccharides.



Kimura *et. al.*[51,52] have synthesised modified 6,6-type polyamides that contain γ-lactone rings and hydrophilic groups resulting from the hydrolytic cleavage of the γ-lactone rings during the reaction. A solution of the bifunctional lactone monomer (2R*, 4S*)-4-chloroformyl-2-chloroformylmethyl-2,4-dimethyl-4-butanolide with adipoly chloride in carbon tetrachloride was stirred at 10,000 rpm. with hexamethylene diamine and an excess of sodium hydroxide (Fig. 1.8). The optimum intrinsic viscosity of the product was 0.85 g dL^{-1} with 15% yield using a quarter of the stoichiometric amount of NaOH as the acid acceptor and benzene as the organic solvent. If the organic solvent was replaced with carbon tetrachloride then the highest intrinsic viscosity obtained was 0.76 g dL^{-1} with a more acceptable yield of 35% using the same amount of acid acceptor.

The best yield in carbon tetrachloride was obtained using sodium hydroxide in a stoichiometric amount as the acid acceptor. The initial ratio of open to closed rings is between 40 and 50 % in all cases. Chemical treatment of the resulting polymer with 0.6% KOH increased the ratio of open to closed rings to 0.75. Treatment with formic acid, HCl and NH_3 caused backbone amide cleavage in addition to ring cleavage.

Fig. 1.8 Interfacial synthesis of 6,6-type polyamides containing both γ-lactone rings and hydrophilic groups.



One of the advantages of interfacial polycondensation is its versatility and the ability to produce high molecular weight product over a wide range of conditions. Sokolov[14] states that the successful interfacial polycondensation relies on the diffusion retardation of two highly reactive di-functional compounds. This is generally brought about by the interfacial boundary of two immiscible liquids but can also be achieved by a liquid-gas interface[15,14]. The gas phase process described by Sokolov consists of bubbles of a volatile acid chloride such as oxalyl chloride carried in an inert gas such as nitrogen being streamed through an aqueous phase of diamine and acid acceptor. The partial pressure of the acid chloride must be maintained below its vapour pressure at the reaction temperature to prevent condensation. Under a steady flow of nitrogen the amount of acid chloride can be increased by raising the temperature in the evaporator.

The process has proved useful for the synthesis of poly (oxamides) which cannot be made by the liquid/liquid interfacial reaction because of the rapid extraction of the acid chloride into the aqueous phase and subsequent loss by hydrolysis. Increasing reaction temperatures in the gas phase procedure reduced the solubility of the acid chloride in the aqueous phase and so reduced hydrolysis. Sokolov argues that the reaction cannot take place by diffusion of the diamine to the organic phase since there is no organic phase and that the reaction must, therefore, occur in the growing polymeric film. He further states that this may well be the case in liquid/liquid systems and that the two are just modifications of the same heterogeneous process. The importance of high interfacial tension between phases is mentioned and this is supported by the successful use of organic solvents, such as *n*-octane, in place of the aqueous phase in gas phase reactions. Other gas phase interfacial syntheses have employed carbon suboxide or phosgene as the gaseous monomer. Carbon suboxide is a highly reactive diketene which reacts with diamines to give polyamide derivatives of malonic acid. Its low boiling point (6.8°C) makes it useful for the gas phase process although in most cases a solvent, typically toluene, is used.

In the gas phase reaction an increase in molecular weight is seen with increasing temperature up to 50°C beyond which an inflection point is reached and the molecular weight decreases. The decrease is attributed to an increase in hydrolysis between 60 and 70 °C. Phosgene can be used as a gaseous reagent in the interfacial synthesis of polycarbonates from bis-phenols, but it is normally dissolved in an organic solvent.

1.9 CONDENSATION POLYMERS FROM NATURAL METABOLITES

In order to render resulting polymers biocompatible, natural metabolites seem an obvious choice as monomers. Multi-functional natural metabolites, such as amino acids and acids from the metabolic pathways of the Krebs cycle, have been used to synthesise functional polymers showing good biocompatability of both the polymers and their degradation products.

Amino acids such as lysine and ornithine are attractive as monomers for interfacial polymerisation since they possess two active amine groups. Lysine in particular has been used as a monomer in interfacial polycondensation, though not necessarily with a view to biomedical application. Saotome *et. al.*[25] described the synthesis of an optically active polyamide based on lysine and adipoyl chloride. The importance of a regular backbone sequence for the comparison of an optically active polymer with its inactive analogue was noted. It was further pointed out by Crescenzi *et. al.*[57], that structural regularity was necessary for crystallisation of the polymeric chains potentially giving rise to interesting spectral properties. Two different approaches for achieving structurally regular polymers were taken by the two groups. In the first case, a symmetrical diamine was produced by selective protection of the α-amino group of lysine using copper acetate and reaction with adipoyl chloride. After de-protection, the symmetrical diamine was polymerised with adipoyl chloride in a CCl_4 /aqueous NaOH system (Fig. 1.9). Intrinsic viscosities were around 0.09 dLg^{-1} (methanol, 25°C) were obtained. Alternatively, lysine diketopiperazine was polymerised with adipoyl chloride in a similar system (Fig. 1.10) to give a white solid with a 68% yield and intrinsic viscosity of 0.21 dLg^{-1} (trifluoroethanol + 4% v/v trifluoroacetic acid, 25°C).

Ihara *et. al.*[50] synthesised a range of optically active polymers from lysine and the diacid chlorides of adipic acid, *tera*-phthalic acid, 4,4-diphenyldicarboxylic acid and 2,6-naphthalenecarboxylic acid by interfacial polymerisation. They investigated the asymmetric interaction of the polymers with α-amino acids, including tryptophan to test the feasibility of using such polymers for the resolution of α-amino acids on synthetic polymers following reports of such separations on natural biopolymers such as wool.

Fig. 1.9 Interfacial polymerisation of polyamides with regular structural sequences from α-amino acids.

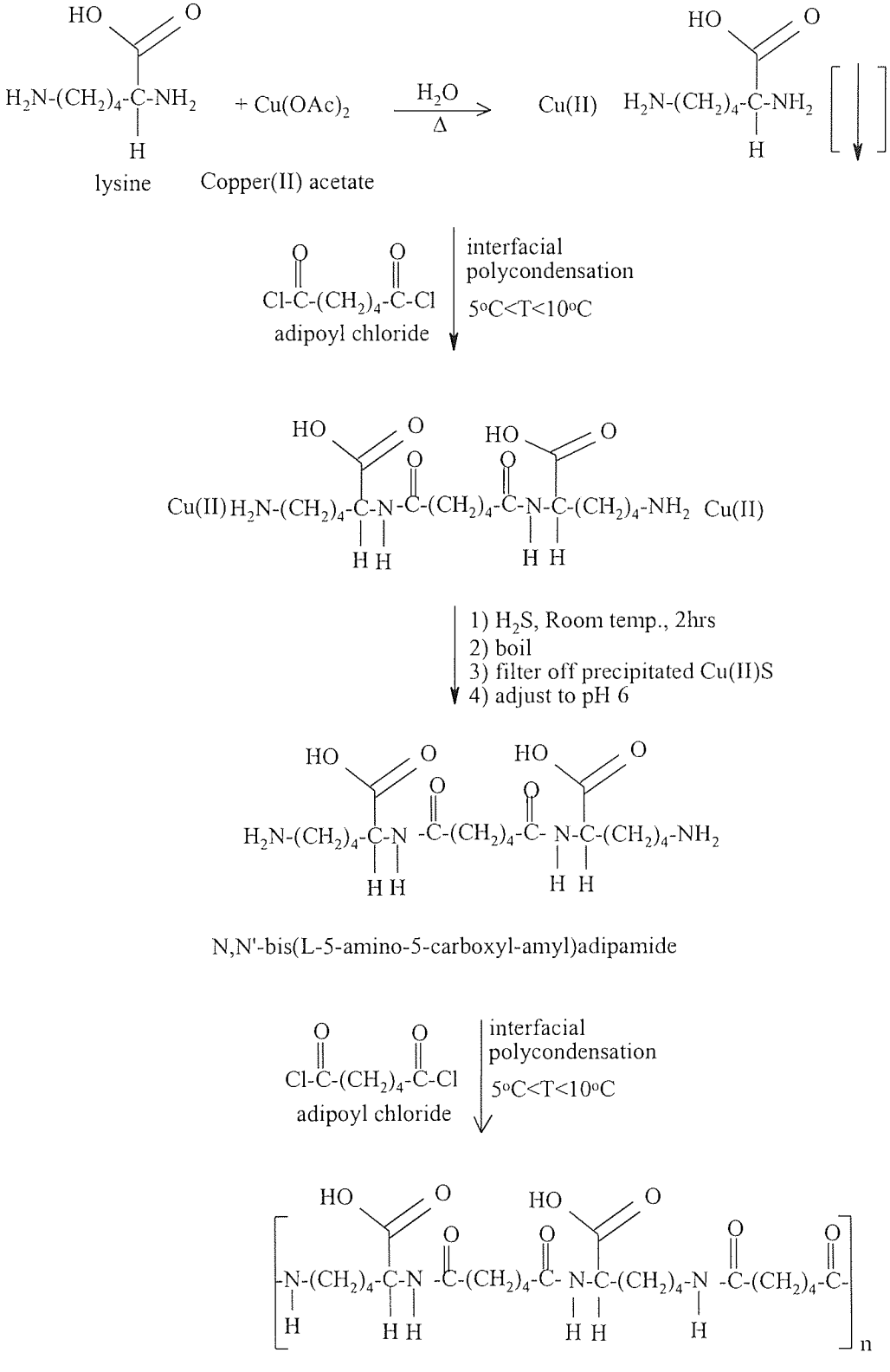
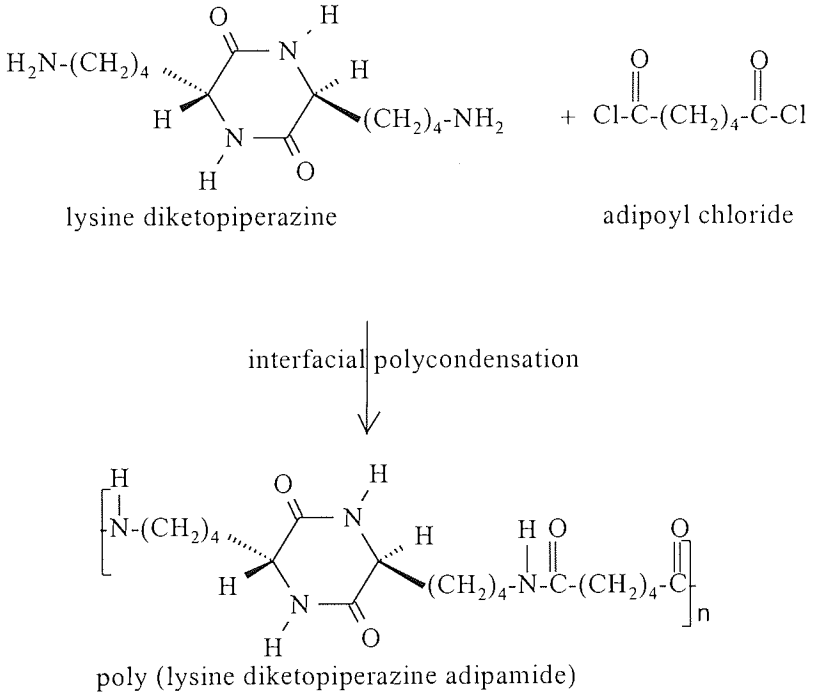


Fig. 1.10 Interfacial polycondensation of lysine diketopiperazine with adipoyl chloride.



The amphoteric nature of lysine means that inactive zwitterion formation occurs at pH values below the pKa of the α-amino group. Maintaining a sufficiently high pH throughout the reaction would avoid this, however, hydrolysis of the diacid chloride would inevitably increase. Esterification of the carboxyl function group prevents zwitterion formation and simultaneously increases the partition coefficient of the diamine into the organic solvent relative to the charged free acid. In addition, free base lysine alkyl esters are unstable and prone to cyclisation and self condensation so should not be stored for long periods of time. N^{α},N^{ε}-bis-(trimethylsilyl) derivatives[58,59] are reportedly more stable under storage and are easily purified by vacuum distillation. The dihydrochloride salts of the free esters are sufficiently stable below 0°C to allow storage and the free base is generated under the alkaline conditions required for an interfacial reaction. The polycondensation reaction proceeds at a sufficiently high rate to avoid the above mentioned side reactions as is evidenced by the successful synthesis of several polyamides from lysine alkyl and aryl esters by the interfacial technique[43,44].

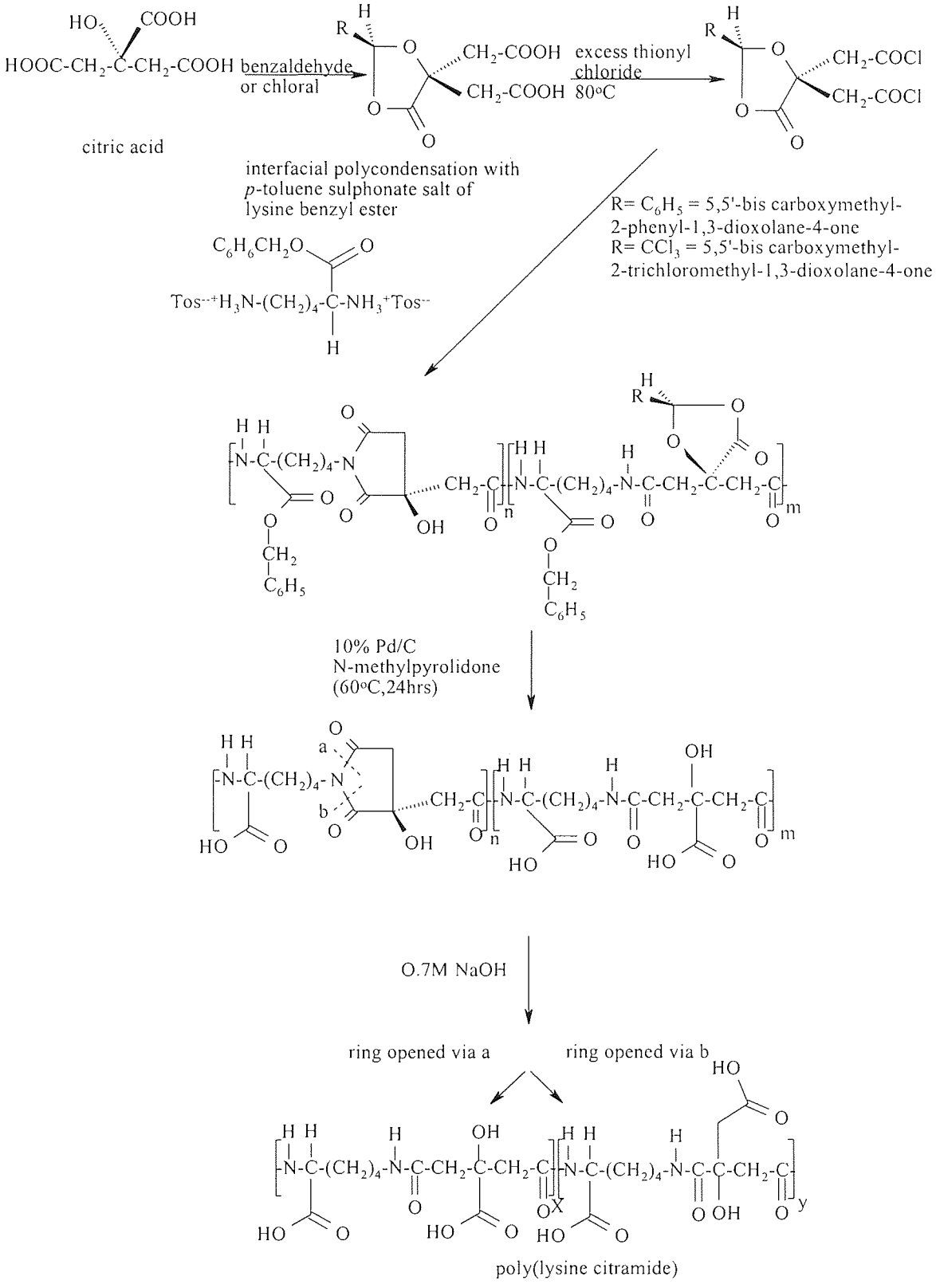
The synthesis of polysulphonamides from lysine and 1,3-benzene disulphonyl chloride using a CH_2Cl_2/aqueous sodium carbonate two phase system was reported by Beaumis *et. al.*[60]. In fact lysine ethyl ester is used in the polymerisation and the ester groups are subsequently hydrolysed back to free carboxyl groups. The polysulphonamides with pendant carboxyl groups exhibits a conformational change from a compact hypercoiled state at low degrees of ionisation, to an extended polyelectrolyte structure at high degrees of ionisation[61]. The compact state is stabilised by hydrophobic interaction of the asymmetric benzene groups but as the degree of ionisation increases electrostatic repulsion between the pendant carboxyl groups causes an extension of the backbone. This type of conformational change is seen in vinyl polymers such as polymethacrylic acid and hydrolysed polystyrene maleic anhydride but is not reported in biocompatible polymers, however, the more common conformational change from an alpha helix to an extended chain seen in poly (amino acids) has been widely investigated.

Vert *et. al.*[43,44] used a similar approach in the synthesis of hydrosoluble polyamides from citric acid and lysine. Citric acid was partially protected via intramolecular cyclisation of the α-hydroxy and α-carboxyl groups to form an oxa-lactonic ring. This was then converted to the diacid chloride derivative with thionyl chloride and polymerised interfacially with lysine-benzyl ester ditosylate using a benzene/aqueous alkali system. The maximum molecular weight of the resulting polymer was 20,000Da (GPC. dioxane w.r.t. polystyrene standards). FT-IR analysis revealed that cyclic imide rings (>C=O at 1710, 1780 cm^{-1}) had largely replaced the oxa-lactonic rings initially present (>C=O at 1820 cm^{-1}). This is attributed to hydrolytic cleavage of the oxa-lactonic protecting function under the alkaline conditions of the reaction, followed by reaction of the hydroxy acid group with the amide bond in the backbone to give an imide ring. Subsequent alkaline hydrolysis of the imide rings leads to structural isomerism within the backbone resulting from two different sites of ring opening (Fig. 1.11). The benzyl ester protecting group on the lysine moiety is removed by a palladium-charcoal catalytic hydogenolysis in N-methyl-pyrrolidone at 60°C.

The resulting polyamide contains both pendant carboxyl functions for covalent attachment of drugs and hydroxyl functions which allow an increased substitution of the carboxyl group whilst maintaining water solubility.

Multi-functional amino acids and derivatives of multi-functional amino acids other than lysine have been investigated. In particular, the work of Kohn *et. al.*[6,7,47,48,62] and Mungara *et. al.*[45,46] on tyrosine containing polymers is of interest. Mungara synthesised tyosine-leucine dipeptides using a standard DPPA peptide coupling technique. N-BOC tyrosine was coupled to leucine with the carboxyl function protected as the methyl ester. The carboxyl group of the leucine moiety was subsequently deprotected and the di-peptide condensed to a symmetrical tetra peptide with hexamethylene diamine using the same DPPA coupling technique. Solution polycondensation of the tetrapeptide in dry chloroform with adipoyl chloride or sebacoyl chloride using triethylamine as the acid acceptor gave white solids with yields of 52.2% and 37% and inherent viscosities of 0.14 dLg^{-1} and 0.13 dLg^{-1} respectively (90% formic acid) corresponding to molecular weights of 14200Da and 20800Da respectively. Interfacial polymerisation of the free tetrapeptide with either diacid chloride gave a white fibrous solid that was insoluble in most organic solvents. The reaction product was shown to be a polyesteramide cross linked through the phenolic hydroxyl groups of tyrosine. If the N-protected tetrapeptide was polymerised interfacially an oligomeric polyester with an intrinsic viscosity of 0.18 dLg^{-1} corresponding to a molecular weight of 3500Da was obtained (Fig. 1.12). FT-IR analysis of the interfacially produced polymers showed absorptions due to ester bonds ($1765cm^{-1}$). These were not present in polymer produced by solution polycondensation. The hydroxyl groups of tyrosine are only reactive towards di-acyl chlorides in highly alkaline medium where the phenoxide ion will be present. These results indicate that under suitable conditions polyesteramides bearing pendant carboxyl groups may be prepared from tyrosine and a diacid chloride.

Fig. 1.11 Synthesis of poly (lysine citramide) from oxolactonic derivatives of citric acid and lysine benzyl ester.



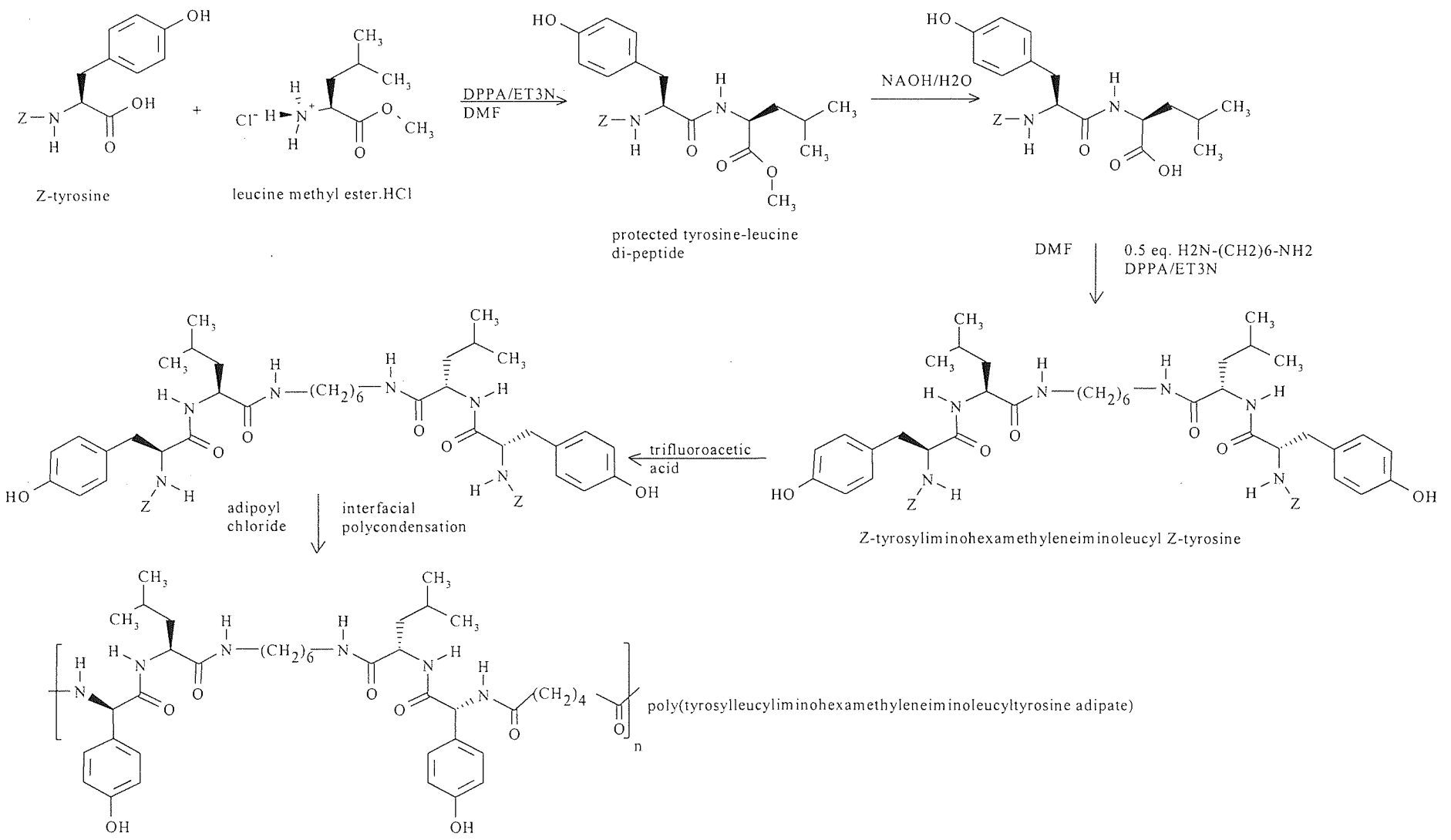
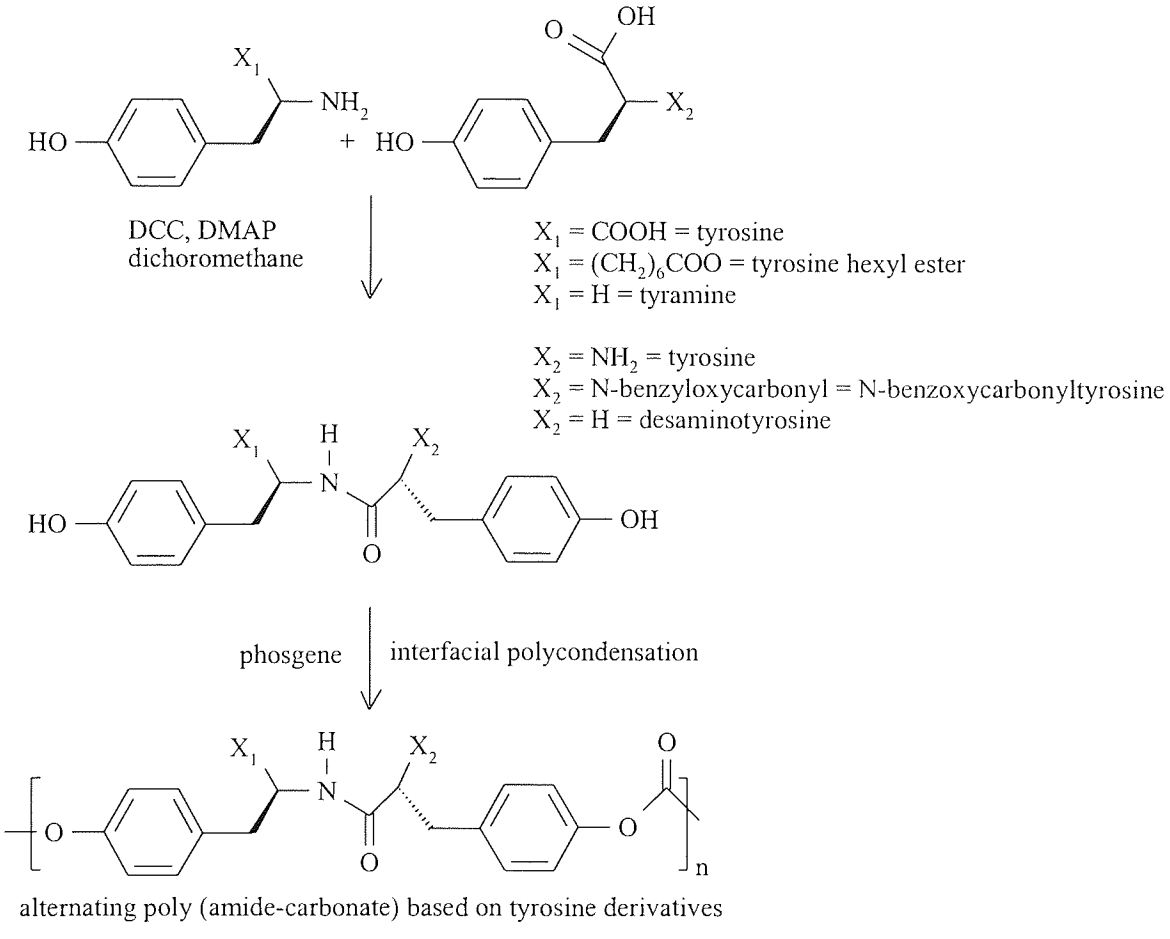


Fig. 1.12 Interfacial synthesis of polyesteramides from tyrosine-leucine dipeptides.

In a similar approach Kohn *et. al*[47,48] synthesised a number of dipeptides based on tyrosine, desaminotyrosine and tyramine and used them to produce pseudo-polypeptides incorporating non-peptide linkages into the polymeric backbone (Fig. 1.13). The inclusion of non-amide backbone linkages reportedly gave polymers with excellent physical properties and good processability. Polycarbonates were obtained with molecular weight of up to 400,000Da (by GPC relative to polystyrene standards), by interfacial polymerisation of the dipeptides in aqueous sodium hydroxide with triphosgene in methylene chloride.

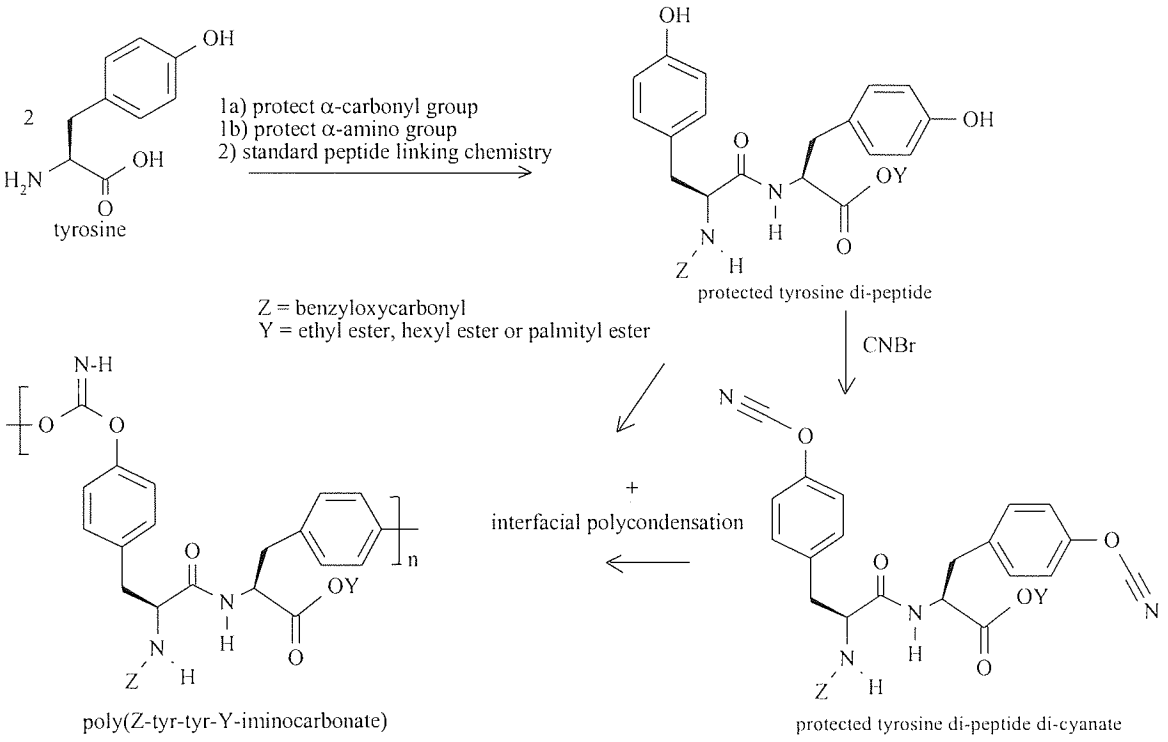
Fig. 1.13 Interfacial synthesis of tyrosine-derived polycarbonates.



alternating poly (amide-carbonate) based on tyrosine derivatives

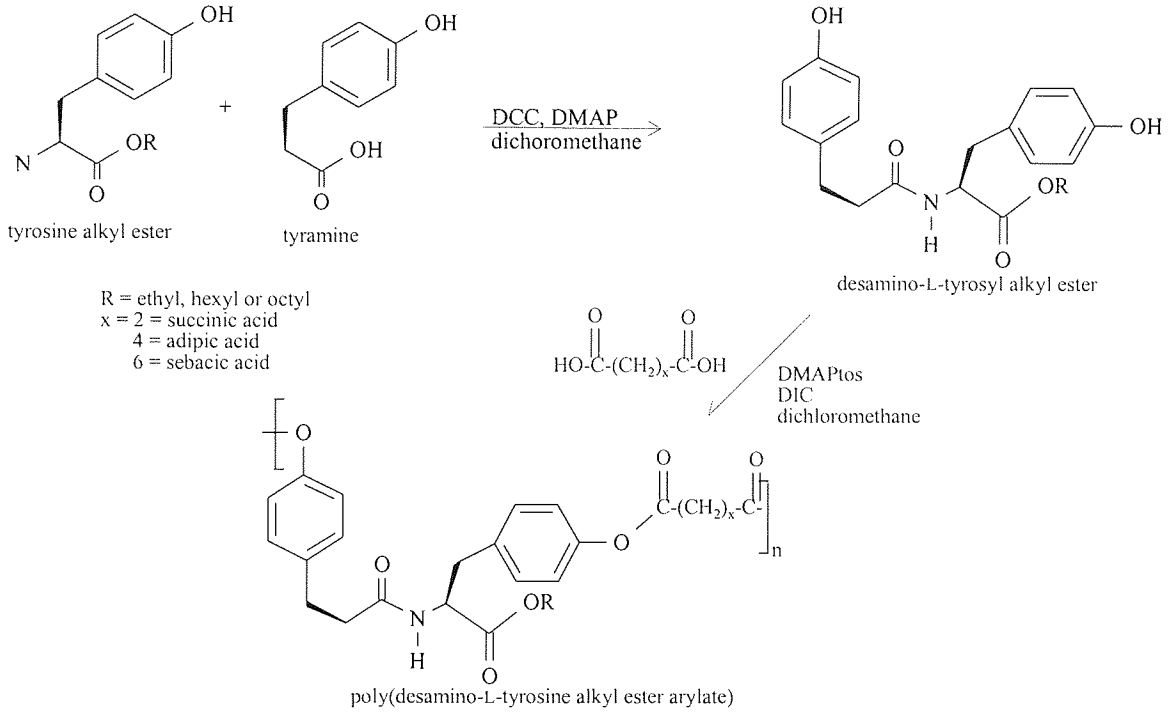
Polyiminocarbonates were synthesised from the protected tyrosine dipeptides in an attempt to obtain biodegradable polymers with engineering properties similar to polycarbonates. The dicyanate derivatives of the dipeptides were polymerised with the diphenol derivatives by solution and interfacial polycondensation. The interfacial system consisted of an aqueous sodium hydroxide solution of the bis-phenol with 10% tetrabutylammonium bromide as a phase transfer catalyst and a methylene chloride solution of the dicyanate (Fig. 1.14). Molecular weights in the range 71,500-175,000Da were obtained (by GPC relative to polystyrene standards). Li[47] pointed out that the interfacial synthesis of iminocarbonates was unique in that hydrolysis of the dicyanate generates the other reagent, i.e. the diphenol. In fact, it was possible to synthesise an iminocarbonate from a solution of bis-phenol A dicyanate in carbon tetrachloride by mixing with an aqueous sodium hydroxide solution containing a phase transfer agent. A polymeric product with molecular weight of 35,500Da was obtained (by GPC relative to polystyrene standards). Alternatively, the polyiminocarbonate can be prepared by the interfacial reaction between bis-phenol in aqueous sodium hydroxide with phase transfer agent and cyanogen bromide in carbon tetrachloride. The reaction only gives a polymeric product if the aqueous phase is added to the well stirred organic phase. If the organic phase is added to the aqueous phase then the cyanogen bromide, which is readily soluble in the aqueous phase, is exposed to a large excess of base and is predominantly hydrolysed. The first method of addition results in the formation of both mono and di-cyanates which accumulate in the organic phase and then react with the bis-phenol, as in the case of the reaction between dicyanate and bis-phenol. The method gives poly (iminocarbonates) with molecular weights in the range 44,000-53,000Da (by GPC relative to polystyrene standards).

Fig. 1.14 Interfacial polycondensation of dicyano derivative of di tyrosine with di tyrosine to form poly (iminocarbonates) based on natural metabolites.



Degradable aliphatic polyarylates have been produced from alkyl ester desaminotyrosine-tyrosine derived dipeptides[7] (Fig. 1.15). A modified version of the solution polymerisation detailed by Moore and Stupp[9] involving the use of condensing agents gave product with molecular weight of up to 230,000Da (by GPC relative to polystyrene standards). A solution technique was used as the conditions for interfacial synthesis were considered too severe for the dipeptides used. Although similar dipeptides were used in the interfacial synthesis of polyiminocarbonates[48], under conditions applicable to polyesterification, these dipeptides did not contain esterified α-carboxyl functions which are susceptible to hydrolysis in basic aqueous solution. Polyesteramides containing tyrosine moieties with pendant carboxyl groups could be synthesised interfacially.

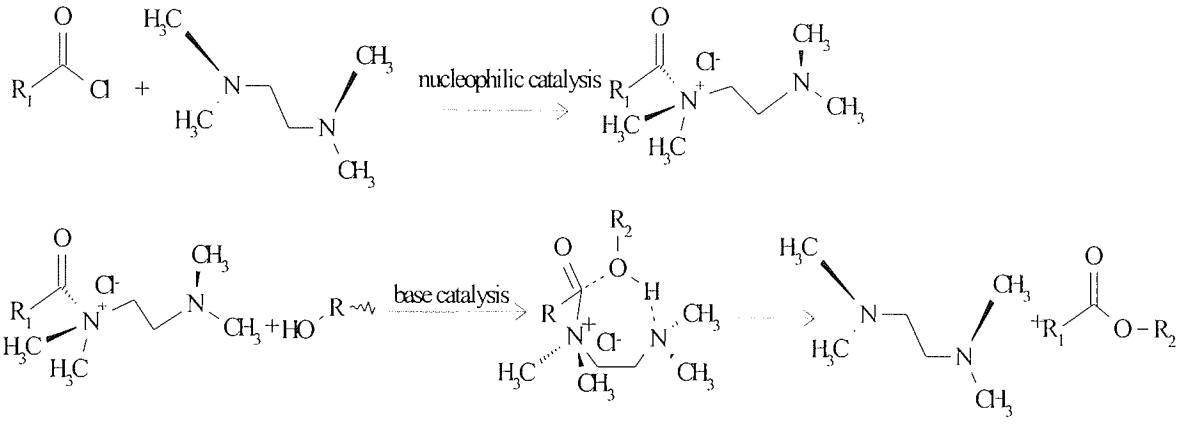
Fig. 1.15 Interfacial synthesis of tyrosine containing polyarylates.



Wang *et. al.*[2] have recently developed a novel organic/organic phase interfacial process for the synthesis of a range of polyesters of *iso*-phthaloyl chloride with functionalised aliphatic diols and their copolyesters with bis-phenols. A low temperature method was required for the chemoselective synthesis of linear polyesters from dihydroxy compounds with additional functional groups. Solution polycondensation gave only low molecular weight product (inherent viscosities typically <0.1 dLg^{-1}), because of the insolubility of the resulting polymers. Conventional aqueous/organic phase interfacial polycondensation using either KOH or TEA as acid acceptor also gave poor results in the synthesis of homopolyester from aliphatic diols containing amino acid moieties because of the relatively higher nucleophilicities of water and hydroxide. With KOH a 15% yield of a product showing characteristic absorptions due to anhydride bonds (1790 and 1850cm^{-1}) was obtained. In addition, the much higher nucleophilicity of phenolate compared to aliphatic diols in an aqueous system precludes co-polyesterification. Using an organic/organic phase interfacial technique the relative nucleophilicity of the aliphatic diols approaches that of the bis-phenol, de-protonation of intermediates is increased by the use of an aprotic solvent and competition from hydrolysis is removed.

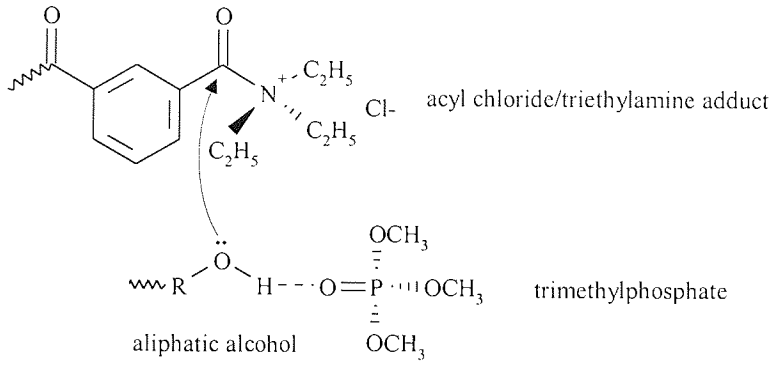
A DMAc/*n*-heptane system was used initially with triethylamine (TEA) as the acid acceptor. TEA was later replaced by tetramethylethylenediamine (TEMED) since the hydrochloride salt of the latter was more easily separated from the resulting polymer by selective dissolution of the polymers in methanol. Higher yields and molecular weights were obtained with TEMED compared to TEA, with optimum ratio of *iso*-phthaloyl chloride to TEMED of 2:1. The increased activity of TEMED was explained by a nucleophilic and basic catalysis of the reaction through an intermediate intramolecular ring structure (Fig. 1.16). This is energetically more favourable than the corresponding intermolecular process involving TEA.

Fig. 1.16 Mechanism of action of N,N,N',N',-tetra-methyl ethylene diamine in the interfacial polyesterification of aliphatic alcohols with aromatic diacyl chlorides.



The increase in heat of reaction and the decrease in interfacial tension caused by the addition of tertiary amines effects the stability of the interface. Replacing DMAc with TMP gives a more stable interface over a wider range of conditions. TMP is also proposed to exert a solvent effect on the hydroxyl group of the diol increasing its nucleophilicity (Fig. 1.17).

Fig. 1.17 Enhancement of nucleophilicity of aliphatic alcohols due to solvent effects.



In further studies, *n*-heptane was replaced by cyclohexane which has a higher partition coefficient for *iso*-phthaloyl chloride. Diffusion of the diacid chloride into the TMP phase was thus reduced in order to avoid the formation of cyclic condensation products. Co-polyesters having ratios of phenolic to aliphatic alcoholic residues approaching one were produced in excellent yield (>90%) with inherent viscosities around 0.41 dLg^{-1}. Copolymers containing both amino acid moieties and pendant quaternary ammonium groups[54] were then used as a novel catalyst, called "polycat", in the aqueous/organic phase interfacial synthesis of aromatic polyesters with pendant carboxyl groups[23]. The chemoselective synthesis of linear polyesters was confirmed by the absence of absorptions due to anhydride linkages in the infra red spectra of the resulting polymers.

The aqueous/organic phase interfacial synthesis of polyarylates generally requires the use of a phase transfer catalyst to transfer bis-phenoxide to the organic phase in order to obtain high molecular weight product. The solubility of the complex formed between the phenoxide ion and the phase transfer agent determines the effectiveness of the agent. If "poly-cat" was used as the catalyst then the inherent viscosity of the product was double that obtained if TBAB was used (0.83 dLg^{-1} cf. 0.41 dLg^{-1}), although both gave yields of approximately 85%. When TEA was added to the system using TBAB the inherent viscosity was reduced to 0.19 dLg^{-1}. This is attributed to the nucleophilic catalysis through complex formation with the acid chloride mentioned previously. The activity of the acid chloride is increased not only towards the phenoxide ion, but also to hydrolysis and anhydride formation by reaction with the carboxylate functions.

Absorptions due to anhydride bonds were detected in the infra red spectra of polymers produced in the presence of TEA. The activity of "poly-cat" is explained in terms of a double transport mechanism. Firstly, the quaternary ammonium group complexes with the phenoxide ion and assists its transport into the organic phase, as with TBAB, but the amino group in the backbone can also complex with the acid chloride assisting its transfer into the aqueous phase where the hydrophobicity of the polymeric segments around the intermediate reduce nucleophilic attack of the carboxylate and water on the carbonyl carbon. The optimum ration of bis-phenoxide to diacid chloride for synthesis of high molecular weigh product is found to be between 0.8 and 0.9. This is explained in terms of the structure of the oligomers formed at the initial stages of the reaction. At this ratio the oligomers with pendant carboxyl groups will have acid chloride groups at both ends and their solubility is reduced in both phases restricting the oligomers to the interface. Increasing the amount of bis-phenol means that the reaction must occur entirely in the aqueous phase. The organic/organic phase interfacial process could equally be applied to the synthesis of polyamides where availability of the diamine in the diacyl chloride containing phase is restricted, because of charged functionalities on the diamine or in cases where hydrolytically unstable acid chlorides are used. One important restriction is the solubility of the diamine in the organic solvent replacing water.

Non stirred interfacial synthesis has been used in the formation of membranes and for coating other fibres. Stirred interfacial polymerisation has been used for the synthesis of microcapsules where a polymeric film is formed around droplets of the aqueous phase[63]. Provided that the stirring does not tear apart the film produced, pharmaceuticals or other active agents can be dissolved or dispersed within the aqueous phase and thus encapsulated by the polymeric membrane. The size of the microspheres produced is controlled by the addition of surfactants and the speed of stirring.

Suzuki *et. al.*[64] produced microcapsules of poly (lysine *tera*-phthalamide) by interfacial polymerisation. An aqueous sodium carbonate solution of lysine was stirred with 10 volumes of mixed organic solvent (chloroform-cyclohexane 1:3v/v) with sorbitan trioleate as an emulsifier. The system was stirred for 10 minutes to give a water-in-oil emulsion. To this, a solution of *tera*-phthaloyl dichloride in the mixed solvent was quickly added.

The microcapsules thus produced were collected by centrifugation and washed with the mixed solvent to remove the emulsifier. Kondo *et-al*[65] have investigated microspheres of poly (lysine *tera*-phthalamide) containing solutions of sheep haemoglobin as artificial red blood cells, since the charge densities of such microspheres were similar to that on red blood corpuscles and both systems exhibit pseudo-plastic flow. In the interfacial synthesis of microcapsules, Kondo *et. al.*[66,67] found that the equilibrium partition coefficient of amino acids that would be expected to be negatively charged under the reaction conditions of a typical interfacial synthesis were considerably higher at low temperature that at room temperature. Thus, the partition coefficient (defined as the ratio of amino acid in the organic phase to that in the aqueous phase) of a 0.4M solution histidine containing 15 %v/v span 85 emulsified with choroform-cyclohexane organic solvent system (1:4 and 1:3) was 0.35 at 30°C and 1.00 at 3°C[67]. Subsequent interfacial polycondensations were carried out in ice baths.

Santo and Abend[68] described the production of biodegradable microspheres from aliphatic polyamides such as poly (lysine adipamide) and poly (lysine sebacamide) for oral drug delivery and Ban *et. al.*[69] reported that poly (lysine sebacamide) was both hydrolytically and enzymatically degraded whereas the rate of enzymatic degradation of poly (lysine *tera*-phthalamide) was found to be the same as the rate of hydrolytic degradation suggesting no specific enzymatic activity.

2. CHAPTER 2

APPLICATIONS OF HYDROSOLUBLE. FUNCTIONAL POLYMERS IN DRUG DELIVERY

2.1 INTRODUCTION

Oral administration of drugs offers advantages over intravenous injection such as self administration and patient comfort. However, the bioavailability of orally administered drugs can be limited by enzymatic degradation[70,71,72] or acid lability of drugs, such as erythromycin[73], in the gastric juice. Other complications, include gastric irritation because of contact with the gastric mucous membrane[71,74] (as is seen with aspirin[75]) and the bitter after taste of certain acidic drugs[74,75]. The use of enteric coatings has surmounted many of these problems. Enteric polymers are film forming polyacids that show pH dependent solubilities arising as a result of their chemical structure. A range of such polymers are commercially available[76] and the choice of polymer allows a coating showing a particular pH of dissolution to be applied to the drug. Generally, the coating remains intact during transit through the stomach where the pH is relatively low (typically <2) and becomes soluble in the upper duodenum where the pH increases (pH typically in the range 5-6) allowing dissolution of the drug. Variation in the type of enteric coating allows certain regions of the gastrointestinal tract to be targeted by utilising further changes in pH to instigate solubilisation of the coating. For example, on moving from the proximal to the terminal ileum the pH increases to around 8[74]. Variations in the pH of the gastric fluid can occur under physiological conditions in subjects with hypo- or anacidity or if antacids have been administered[77]. Such variations in gastric pH can result in premature release of drugs into the stomach and thus lead to the associated side effects or loss of bioavailability of the drug. This has lead some authors to investigate enteric coatings that rely on differences in enzymatic activity of the various sections of the gastrointestinal tract to trigger decomposition of the coating and thus drug release rather than the traditional pH triggering mechanism[77,78]. Thus Yoshimoto *et. al.*[77] determined the lag times to dissolution of tablets of a model drug, sulphamethisole, coated with 1% each of ethyl cellulose and various triglycerides in a modified JPXI dissolution test. In the test, coated tablets were exposed to simulated gastric fluid (JP-1 pH 1.2) and to a second fluid simulating conditions on passing to the duodenum (JP-2 pH 6.8). Enteric polymers are generally designed to resist disintegration in the first fluid for over 2 hours and then dissolve in the second fluid.

The ethylcellulose/triglyceride polymers were intended to remain insoluble in the second fluid and dissolve in a third fluid simulating intestinal fluid (JP-2-GL pH 6.7 with a lipase activity of 0.75). Rapid disintegration of the tablet in this third media was required for effective enteric release to the intestine. Whilst no single combination of ethyl cellulose and triglyceride completely satisfied the requirements of an enteric film because of slow release in the third fluid, tablets coated with a combination of ethylcellulose and the triglycerides trimyristin and triolein were found to show more rapid release times in the media with enzymatic activity. The most effective coating formulation was found to consist of 1% each of ethylcellulose, trimyristin and triolein. This coating gave a lag time to dissolution of approximately 30 minutes in the third fluid. The release of the model drug from an ethylcellullose/triglyceride coated tablet in JP-2-GL compared favourably with the release from a tablet coated with commercially available cellulose acetate phthalate enteric polymer in JP-2 fluid. Release of the drug was via a diffusion mechanism accelerated by the degradation of the triglyceride component of the film by enzymatic breakdown[74].

Yeh *et. al.*[79]. proposed the use of a combination of change in pH and enzymatic activity to achieve targeted delivery of peptides or proteins to the colon where the digestive enzyme activity is lower compared to the small intestine. These workers exploited the specific enzymatic activities of the colon to achieve degradation of a pH responsive hydrogel. As the pH of the medium increases, the hydrogel expands as a result of the increasing charge repulsion between weakly charged carboxyl groups (derived from acrylic acid). Expansion of the polymer network allows access of azoreductase to the azo crosslinks of the hydrogel resulting in degradation of the hydrogel network. Degradation of the hydrogel would then lead to release of peptides or proteins trapped within the hydrogel network. Drugs for local colon disease may also be delivered in this way.

2.2 SUSTAINED RELEASE.

In addition to the advantages mentioned in section 2.1 enteric and other coatings can be used to provide a mechanism for sustained release through variation in the gastric emptying times of the dosage form. Variable gastric emptying times were initially seen as a potential problem with singular dosage forms, but this problem is alleviated by the use of multiparticulate dosage forms[72,80,81]. Multiparticulate dosage forms generally consist of a gelatin capsule containing several hundred or even thousands of individually coated milli or microspheres. Non enteric polymers such as ethyl cellulose, which remain insoluble at all pHs encountered along the gastrointestinal tract, are usually applied as coatings to drug particles to achieve sustained release through diffusion[76]. Sustained release dosage forms have been extensively used since the introduction of the Spansule™ range of products by Smith, Kline and French in the early 1950s[74]. Sustained release is achieved through variation of the composition and/or the amount of coating applied to the microparticulates which rapidly spread out along the gastrointestinal tract on dissolution of the gelatin case[74,82]. The result is a more uniform absorption with less patient to patient and dose to dose variation than can be achieved with single dosage forms.

2.2.1 Coating techniques.

The various coating techniques and the effect of coating parameters such as coating formulation and solvent choice are adequately covered in the literature [e.g.[74,76,83]] and so will be only briefly considered in this thesis. Pan coating and fluidised bed spray coating have been widely employed for the coating of microcapsules. Pan coating usually require spheres with diameters of more than 500μm. Fluidised bed coating offers some improvement, but even the most effective Wurster coating technique can only cope with particles with diameters greater than 50μm[76]. Coacervation or phase separation methods are also frequently employed.[82] and will be discussed in more detail in Sec. 2.3.1.

The effectiveness of a particular polymer as either an enteric coating or as a coating to provide sustained release depends on a number of processing parameters as well as the nature of the polymer. The formation of an intact coherent film is vital to the production of coated oral dosage forms that exhibit reproducible release characteristics. Further, the integrity of the film needs to be maintained throughout the storage lifetime of the dosage form and so the mechanical properties and chemical stability of the film are important. Plasticisers are added to the film formulations in order to improve the film formability of the coating polymer and improve the mechanical properties of the resultant coatings during storage. A secondary benefit of coated dosage forms is the reduction in drug instability resulting from contact with atmospheric moisture during storage[84]. Porter *et. al.*[84] investigated the effects of plasticiser content and the presence of the insoluble pigment, red iron oxide, on the permeability of enteric coatings of poly (vinylacetate phthalate) and cellulose acetate phthalate to water vapour. The investigation was intended to give some insight into the reason for the failure of several tested brands of enterically coated aspirin. These workers found significant differences in the permeability of the two enteric coating materials to water vapour. Chemical differences in the structures of the film forming polymers were presumed to be partly to blame. The vinyl backbone of polyvinyl acetate phthalate was reasoned to be less hydrophilic than the cellulose based polymer. The higher water affinity of the cellulose polymer would lead to adsorption of water and increased plasticisation of the film accounting for its higher permeability. Possible physical differences in the film were also used to account for the observed differences in permeability. A solvent system with a higher volatility was used to apply the cellulose acetate phthalate coating, which may have resulted in the formation of a more porous coating with a consequently higher permeability. Increasing the plasticiser content would be expected to increase segmental mobility and, therefore, reduce the activation energy for diffusion. Thus the permeability of poly (vinyl acetate phthalate) increased with increasing plasticiser content. However, in the case of cellulose acetate phthalate the permeability initially decreased sharply with an increase in plasticiser content and then began to rise gradually with further increases in plasticiser content as with polyvinyl acetate phthalate.

The initial rapid decrease in permeability was ascribed to a reduction in the porosity of the cellulose acetate phthalate film because of the solvating effect of the plasticiser on the polymer. The presence of the insoluble pigment caused an initial decrease in the permeability of the film due to a rise in the tortuosity of the diffusion path. Further increases in the amount of insoluble additives eventually exceeded the binding capacity of the polymer causing an increase in the permeability of the film. Similar trends in permeability to liquid water in simulated gastric juice were observed, although the magnitude of the differences between the two polymers was reduced. The reduction in the relative permeability of the cellulose acetate phthalate film was attributed to immobilisation of the diffusant by association at polar sites (which are potentially more numerous in the cellulosic polymer) in the polymer network.

Post hardening of enteric coatings can take place as a result of chemical modification within the film structure. In the case of cellulose acetate phthalate films transesterification between acetate and phthalic acid groups can lead to reduction in permeability of the film because of the formation of crosslinked phthalate ester bridges[5]. Post curing of the films at 65 to 75 °C was found to stabilise the films in an equilibrium state that did not alter with time. Cellulose acetate phthalate films can be chemically stabilised by blending with a basic polymer such as poly (vinylpyrrolidone). Interaction of the pyrrolidone groups with the carboxyl groups prevents crosslinking[5]. Additives are often added to film formulations in order to improve coating efficiency (e.g. anti-tack agents[76,85]) or their appearance (e.g. pigments[76,84]) to allow identification of different batches. Surface roughness and the presence of logo depressions can result in the formation of pinhole defects within coatings. Down[86] proposed that these pinhole defects arose as a result of reduced interparticulate abrasion within the imperfections of the particles as the coating is applied. Interparticulate abrasion rapidly breaks down bubbles on the particle surface from foam formed during the spray atomisation of the coating solution. Surface defects in applied coatings were found, not unsurprisingly, to result in a loss of enteric or sustained release properties.

2.2.2 Surface neutralisation method.

One coating technique not covered in the recent review literature is the surface neutralisation method[71,75,89,87,88]. By this method, particles of a sparingly soluble acidic drug are dispersed in an aqueous solution of enteric polymer solubilised with the minimum amount of alkali. The sparingly soluble acidic dug forms a saturated solution in the vicinity of the particle surface causing a localised drop in pH. The enteric polymer precipitates at the surface of the particles and forms a seamless coherent uniform film. Koida *et. al.*[87] showed that the thickness of the coating can be varied with the amount of enteric polymer in solution. Increasing the film thickness of carboxymethyl ethyl cellulose coated aspirin up to 4μm was found to increase the enteric properties of the particles (i.e. increased dissolution times in simulated gastric juice) whilst further increases reduced the effectiveness of the coatings. This unusual observation was explained on examination of the films by SEM. Cracks, assumed to result from shrinkage during drying were visible in the thicker films. The effect of encapsulation temperature was also studied. As the temperature was increased the membrane thickness increased causing a reduction in the release of encapsulated aspirin. Increasing the temperature increased the solubility of the aspirin in the microencapsulation medium and may lower the pH around the crystals to a greater extent than at lower temperatures resulting in thicker wall formation. Increased dissolution of the aspirin resulted in a lower microcapsule content of the drug at elevated temperatures. Takahata *et. al.*[71]. extended the method to the encapsulation of non-acidic drugs by coating core granules of a sparingly soluble drug containing organic acids such as maleic or fumaric acid. Thus indomethacin, a sparingly soluble non acidic drug, known to cause irritation of the mucous membrane, was powdered with maleic or fumaric acid, kneaded with 10% of a binder solution (10% ethylcellulose or poly (vinyl alcohol) in 80% alcohol) and extruded to form granules. The granules were sieved through 28 mesh (590μm) and 60 mesh (250μm) screens. Particles with an average diameter of 450μm were used for coating.

Carboxymethyl ethyl cellulose was used to coat the microparticles since other enteric polymers investigated caused coagulation during the microencapsulation process[75]. The carboxymethyl ethyl cellulose content in the microcapsule was found to increase linearly with the amount of maleic acid indicating that the wall thickness can be readily controlled by variation in the organic acid content of the granules to be coated. The wall thickness was also found to be dependent on the encapsulation temperature and the nature of the organic acid. Increasing the temperature resulted in an increase in film thickness with both organic acids, but thicker films were produced with 20% maleic acid at 25°C than with 20% fumaric acid at the same temperature. The film thicknesses were almost equal at 40°C and the differences were attributed to solubility differences between the two acids (fumaric acid being only 50 % soluble compared to 100% solubility of maleic acid at 25°C). Although maleic acid was found to be completely soluble over the temperature range studied, the film thickness still varied with temperature. This was attributed to the increased rate of dissolution and thus accelerated neutralisation of the enteric polymer at higher temperature. Alternative explanations offered were increases in the cohesive force of the polymer for the cores at higher temperatures or a reduction in the amount of acid required to produce a phase transition at higher temperatures because of a reduced affinity of the hydrophilic groups for water at a higher temperature. Increasing the temperature of encapsulation increased the release rates of the drug in both simulated gastric juice and in fluid simulating the conditions of the duodenum. These results were accounted for by changes in the surface morphology of the microcapsules. Increasing temperature caused increased surface roughness and cracking so increased release of drug was seen despite increased enteric film content. The nature of the binder used in granule preparation was found to effect the enteric properties of the microcapsules. When relatively hydrophobic ethylcellulose was used as the binder, penetration of the cores by water and dissolution of the organic acid was reduced, resulting in thinner film formation compared to when hydrophilic poly (vinyl alcohol) was used.

Takahata *et. al.*[89] found that on comparing structurally similar polymers such as hydroxypropylmethylcellulose acetate succinate-L and -H (HPMCAC-L or -H) or Eudragit L and S, thicker coatings were produced from polymers with a higher pH of minimum solubility (pH_{min}). This relationship did not apply when structurally dissimilar polymers were compared. For example hydroxypropylmethylcellulose acetate succinate-H films were thicker than those prepared from Eudragit S despite having an identical pH_{min} of 7.0. In cases of polymer dissimilarity, molecular weight, monomer properties and the high order structural properties of the specific polymer must be considered. Eudragit S coated particles did not exhibit enteric properties and this was attributed to the poor film forming properties of the methacrylic acid based polymer at low temperatures. Eudragit L or S requires large quantities of plasticiser for application by spray coating because of its relatively high film-forming temperature[90]. Takahata *et. al.*[88] investigated the effect of plasticiser content on the film formability of Eudragit S using the surface neutralisation method. Films that gave enteric properties were formed at temperatures over 60°C with 20% triethylcitrate and at temperatures over 40°C with 40% triethylcitrate. These results initially seemed consistent with those of Lehmann[90] which indicated that 20% triethylcitrate lowered the minimum film forming temperature of Eudragit S to 60°C in the spray coating process. However, the plasticiser content of the film deposited by spray coating is expected to approximate that in the spray, whereas in the surface neutralisation method not all the polymer and plasticiser are necessarily used in film formation. It was shown that the plasticiser content in films formed from solutions containing 40% triethylcitrate only contained around 10% triethylcitrate. The plasticising effect of water during the neutralisation process was thought to account for the reduced film forming temperature of the Eudragit S in films formed by the surface neutralisation method. Higher plasticiser contents are required to reduce the film forming temperature in spray coating.

2.2.3 Matrix formulations.

Oral drug dosage forms have been extended from simple tablets to coated tablets and to multiparticulate coated tablets for targeted and/or sustained release. Modification of the release behaviour can also be achieved by forming solid dispersions of the drug with insoluble[90,91], enteric[91-96] or completely soluble polymers[97,98] or monomeric excipients[99-104] depending on the application. The solid dispersion dosage form was originally developed to increase the solubility and thus bioavailability of poorly water soluble drugs[104] by increasing the available surface area of the drug for dissolution. Such matrix or solid dispersion formulations can be administered as single solid dosage forms or as multiparticulates contained within a gelatin capsule. Multiparticulate systems utilising an enteric polymer as the solid dispersant offer the additional advantage of sustained delivery resulting from the spreading out effect of transit through the stomach of the numerous particles. When the first particles pass through the stomach to the duodenum the enteric polymer dissolves causing a greatly enhanced dissolution of the drug. Subsequent particles passing through the stomach maintain the supply of drug. The maximum period of sustained release is, therefore, dependent on the gastric emptying time which will vary from patient to patient[71,94]. For such applications rapid dissolution of the enteric polymer is advantageous since this allows for a maximum rate dissolution of the active component and thus maximum bioavailability. Hydroxypropyl methylcellulose acetate succinate is reported to dissolve more rapidly than hydroxypropyl methylcellulose phthalate and so may be a more suitable choice for the solid dispersant[95]. Rapid dissolution of the enteric polymer and dissolution of blended drug is also desirable in cases where enzymatic action in the lower intestine may lead to loss of bioavailability or in cases where large bowl complaints must be considered.

2.2.4 Limitations of sustained release.

Although significant advances have been made in the field of sustained release from oral dosage forms, not all drugs are suitable for administration by such a mechanism. Sustained release is ideally suited to the administration of antibiotics[95]. Evidence from *in vitro* and *in vivo* work has shown that the efficacy of β-lactam antibiotics depends on the duration of exposure of the bacteria to concentrations of the antibiotic above the minimum inhibitory concentration. Thus any technique that can maintain the level of antibiotic at such a concentration, rather than produce a peak and trough pattern seen with conventional administration, will increase the efficacy of the treatment. Good *in vitro* results from sustained released formulations as solid dispersions with hydroxypropyl methylcellulose acetate succinate failed to live up to expectations *in vivo*[95]. Poor absorption of amoxicillin from the distal jejunum and ileum compared to the duodenum and proximal jejunum, drug inactivation by β-lactamases and incomplete drug release were offered as potential reasons for the poor *in vivo* performance. Drugs preferentially absorbed at particular regions of the gastrointestinal tract are unsuited to delivery systems designed to provide sustained release by diffusion through an insoluble coating such as ethyl cellulose. Vitamin B_2 and iron are absorbed preferentially in the duodenum and so release further down the gastrointestinal tract is of no benefit. Drugs that are extensively metabolised during absorption from the gut or by first by-pass effects are also unsuited to sustained release formulations. Sasahara *et. al.*[72] found that the bioavailability of levodopa in dogs was significantly lower when administered orally compared to other routes (intravenous and hepatoportal), due to extensive metabolisation by high concentrations of dopa decarboxylase, in the intestinal wall.

2.3 CONTROLLED RELEASE SYSTEMS FOR PERITONEAL ADMINISTRATION.

In the context of drug delivery, controlled and sustained release are often taken to mean the same thing, i.e. some form of extended release formulation. There may be some inherent form of targeting associated with such release forms such as the enteric release to the duodenum or colon discussed in Sec 2.1. In this thesis controlled release will be used to describe the extended release from colloidal carriers including microparticulate, nanoparticulate, liposomal and micellular vehicles designed for interperitoneal, intravenous or subcutaneous administration and is generally in the order of weeks to years compared to sustained release oral dosage forms which are usually in the order of hours. Microparticulate carriers can be subdivided into microspheres and microcapsules. Microspheres are defined as solid monolithic polymer particle which may contain drug particles dispersed throughout the bulk and microcapsules are described as a reservoir-type particulate consisting of a polymeric membrane surrounding a distinct solid or liquid core. These definitions are consistent with those previously adopted within the Speciality Materials Research Group[e.g.[83]].

2.3.1 Microparticulate controlled release formulations.

The fabrication of microparticulate drug carrier release vehicles and the mechanisms of drug release are adequately covered in the literature [e.g.[74,76,83]] and so will be only briefly considered in this thesis. Microparticles can be produced by several methods and the choice of method is dependent on factors such as architecture of the delivery device (microsphere or microcapsule), stability of the drug under the processing conditions (e.g. heat or solvent incompatibilities), release characteristics of the device (permeability, porosity), scale-up and batch reproducibility. The fabrication techniques can be divided into two broad categories, i.e. interfacial methods and desolvation. Interfacial methods involve the formation of the microparticulate architecture during the synthesis of the constituent polymers. This can be achieved by emulsion or suspension polymerisation or by interfacial polycondensation. The first two methods produce microspheres whereas interfacial polycondensation generally produces microcapsules.

Emulsion and suspension polymerisation are used in the polymerisation of vinyl monomers and so are not applicable to the production of biodegradable microparticles. Interfacial polycondensation, however, can be used to synthesise a range of potentially biodegradable polymers as discussed in Ch.1. Desolvation techniques are widely employed in the synthesis and coating of microspheres. Typical methods of desolvation include spray drying and phase separation or coacervation which can be achieved by solvent evaporation or solvent extraction. Solutions of polymer and drug can be spray dried to produce microspheres or prefabricated microspheres can be spray coated to produce microcapsules. Both organic and aqueous solutions can be employed with modern spray coating equipment[76]. Agglomeration of particles, production of non-spherical particles, fibre formation at the spray nozzle with film forming polymers and potential deactivation of heat sensitive peptides and proteins are the major problems encountered with the spray drying process[79]. More common approaches to the desolvation approach include coacervation or phase separation. Simple coacervation involves the desolvation of a hydrophilic colloid such as gelatin or acacia by the addition of an agent with a higher affinity for water such as alcohol or inorganic salts. As the amount of water bound by the colloid is decreased, phase separation occurs and the dehydrated polymer molecules coagulate with each other to form the coacervate. Complex coacervation is achieved by the dehydration of one hydrophilic colloid (such as gelatin) by another hydrophilic colloid with an net opposite charge (such as acacia) Charge neutralisation results is a loss of bound water. Coacervation may also be achieved from organic solvents by addition of a non solvent. This extends the coacervation technique to the production of aqueous reservoir-type microcapsules. The encapsulation of water soluble peptides and macromolecules with polylactide/glycolide copolymer as an example of such a process[76]. Addition of silicone oil to an organic solution of polylactide/glycolide copolymer with a dispersed aqueous solution of drug caused coacervation of the lactide/glycolide copolymer and encapsulation of the aqueous droplets. Phase separation techniques involve the removal of solvent from a solution of the polymer and drug (the drug may also be dispersed as a solid) emulsified in an immiscible continuous phase.

This may be achieved by the application of heat (and vacuum) or by the addition of a non solvent that is miscible with both the continuous and dispersed phases. The dispersed solvent is then extracted into the bulk resulting in phase separation of the polymer. Both microspheres and microcapsules can be produced in this way. Microcapsules are produced by first dispersing an aqueous solution of the polymer and drug in an organic phase and then dispersing the resulting emulsion into another aqueous phase to give an water in oil in water dispersion. Removal of the organic solvent leaves reservoir-type water/drug microcapsules[79].

Controlled release from microparticles intended for peritoneal delivery is achieved in one of three ways[105,106]. The first mechanism involves diffusion or dissolution of the drug through the matrix of a microparticle or through the semipermeable membrane of a microsphere (e.g. polycaprolactone and albumin as biodegradable systems or non degradable polysiloxane polymer systems). The second involves release as the polymer matrix, containing entrapped drug, degrades or through chemical cleavage of the drug from the polymer (e.g. polyanhydride and polyorthoester systems). Finally in solvent activated systems osmotic pressure or swelling of the polymer matrix is used as the driving force for drug delivery. In addition to drug delivery, microparticulates and liposomal controlled release systems are receiving attention as antigen release systems for immunisation. Biodegradable polymers are particularly attractive for such an application since their degradation products can be selected to have adjutant properties. The delivery device would then exhibit immunostimulating properties whilst simultaneously delivering antigen over an extended period[105,107]. Tyrosine containing iminocarbonates of the type proposed by Kohn *et. al.*[47] and discussed in Ch.1 have been shown to display adjuvant properties independently of a sustained release effect. Iminocarbonates are biodegradable and so may also serve as extended delivery vehicles.

2.3.2 RES avoidance and drug targeting.

When colloidal carrier systems, such as microparticles or liposomes, are injected into the bloodstream, they are rapidly phagacytocised by macrophage cells in the spleen and the Kuppfer cells of the liver[108,109,110]. Sequestration of microparticles by the cells of the reticuloendothelial system results in the rapid clearance of colloidal particles from the systemic circulation usually in a matter of minutes[108]. This process is useful for targeting delivery of drugs to the organs of the RES, but is a major disadvantage when attempting to target delivery to other organs[111], leading to an unwanted accumulation of the administered particles within the lungs, liver, spleen and bone marrow[110]. This can lead to localised toxicity and reduction in any controlled release effect. In order to target drug delivery from colloidal carriers it is, therefore, necessary to prevent or reduce their uptake by the RES. One simple approach used by Davis *et. al.*[108] in animal studies was to saturate the RES with a placebo dose prior to administration of an active dosage form[108]. This effect was not deemed suitable to use in clinical studies with humans. Oku[111] described a similar effect in a review of long-circulating liposomes, but the effect was seen to be minimal when empty liposomes were administered 24 hrs before administration of loaded liposomes. Tabata *et. al.*[112] investigated the effect of particle size and surface charge on the *in vitro* phagocytosis of hydrophilic cellulose and hydrophobic polystyrene and phenylated polyacrylein microspheres by peritoneal macrophages. These workers found that the maximum number of microspheres phagacytocised was highest at a particle size between 1.0 and 2.0 μm with polystyrene and phenylated polyacrolein spheres. The number of engulfed spheres was significantly reduced in the presence of 10% fetal calf serum, but maximal phagocytosis of particles occurred within a similar size range. The reduction in phagocytosis of hydrophobic microspheres in the presence of fetal calf serum was attributed to a reduction in the surface hydrophobicity by adsorption of albumin. The phagocytosis of surface modified cellulose microspheres with diameters less than 2.0μm was found to be dependent on the hydrophobicity and zeta potential (a measure of surface charge density) of the modified surface. In a comparison of surface modified cellulose microspheres, benzyl-cellulose microspheres were most susceptible to phagocytosis whilst unmodified, non ionic, hydrophilic (lowest zeta potential) were found to be least susceptible.

Both anionic and cationic microspheres showed enhanced phagacytosis compared to the non charged hydrophilic microspheres, but reduced phagacytosis compared to the hydrophobic microspheres. The negatively charged membrane and the presence of divalent cations such as Ca^{2+} and Mg^{2+} was considered an important factor in the phagacytosis of charged microspheres. The lowest rate of phagacytosis was postulated to be for microspheres with a zeta potential of zero. Davis *et. al.*[108] pointed out that phagocytosis of microparticles is often preceded by adsorption of proteins or glycoproteins (opsonisation) and may be prompted by such processes. Understanding the salient features colloidal phagocytosis, principally the nature of the surface in terms of structure and charge, has prompted the modification of microparticulate surfaces to confer the particles with stealth characteristics making them non-recognisable to the host's immune system. Surface modified colloidal carriers (including liposomes) fall into the general category of biomimetic materials. Such materials attempt to mimic the natural protection to phagocytosis afforded to some micro-organisms by hydrophilic oligosaccharide chains present at the cell surface. These chains provide a steric barrier to interaction with the blood components including macrophages and plasma proteins involved in opsonisation. Similar approaches have been used to improve the biocompatibility of biomaterials used for implants and contact lenses[113] and such methods have been investigated in these laboratories. Davis *et. al.*[108] found that polystyrene nanoparticles (diameter 60nm) were directed principally to the organs of the RES whilst those with an adsorbed layer of the tetronic surfactant poloxamine were distributed throughout the body (as evidenced by γ-scintiscans) whereas those coated with the pluronic surfactant polaxamer were preferentially targeted to the bone marrow. It was suggested that the reason for this specificity in localisation of the nanoparticles was due to the adsorption of blood components that conferred the particles with the necessary recognition responsible for delivery to the bone. Coombes *et. al.*[109] Reported a method for the simultaneous production and surface biodegradable modification of microspheres of poly (lactide-co-glycolide) polymers (PLG) with the pluronic surfactant polyethylene oxide-polypropylene oxide using a modified solvent extraction/evaporation process.

Microspheres produced using solvent extraction or solvent evaporation techniques use small amounts (typically around 1%) of surfactant to produce a stable emulsion prior to removal of the solvent[83]. These workers found that modifying the surfaces of pre-synthesised microspheres prepared using poly (vinyl alcohol) (PVA) as the surfactant was difficult since PVA molecules adsorbed to the surface impeded adsorption of the pluronic polymer. Muller *et. al.*[114] overcame this problem by using the ionic surfactant, sodium dodecyl sulphate to increase the surface hydrophobicity of polyester nanoparticles before coating them with poloxomer or poloxamine 908. Successful coating was achieved using poloxamine 908. Coombes *et. al.*[109] reasoned that if the surface coating could be applied during the manufacture of the microspheres then the coating would show improved cohesion with the microsphere's surface and would be less prone to competitive desorbtion in the presence of blood components. Competitive desorbtion of adsorbed coatings, intended to stabilise colloidal carriers in the biological milieu, is recognised as a significant problem. PLG microspheres were successfully prepared by emulsifying 10mL of PLG in a 1:1 mixture of DCM and acetone with 20mL of an aqueous 10%(w/v) PEO-PPO stabiliser. Stirring was continued for 5 minutes following the gradual addition of the PLG solution to the homogenised aqueous solution of stabiliser. The emulsion thus formed was then stirred with a magnetic follower to effect solvent evaporation. The microspheres thus produced had a relatively high polydispersity although this could be reduced by increasing the viscosity of the stabilising solution and reducing the concentration of the PLG solution. It was reasoned that rapid extraction of the acetone into the continuous phase would result in precipitation of the polymer at the surface of the droplets causing entrapment of the surfactant molecules by physical chain entanglement. The droplet size would be reduced and the droplet viscosity would be increased by removal of the acetone, both processes which would be expected to enhance the entrapment of the PEO-PPO chains. When DCM alone is used as the solvent, the rate of increase in viscosity, precipitation of the polymer and droplet contraction would be slower, reducing the efficiency of chain entanglement. In addition, the lower viscosity of the droplets allows stabiliser molecules to diffuse readily into the aqueous phase with the resultant lowering of solvent capacity.

The ratio of PEO to PPO in the stabiliser was found to be important. The hydrophobic PPO chain segments adsorb to the surface of the microspheres whilst the hydrophilic PEO segments provide the steric barrier to phagocytosis. Reducing the PPO chain length reduced the efficiency of stabilisation of the semisolid and produced microspheres with a lower surface coverage. Increasing the length of the PEO chain may result in the adoption of a looped conformation of the hydrophilic PEO chains at low surface coverage.

Similar relationships between size and recognition by the RES apply to liposomal carriers[111]. In addition, rigid liposomes showed longer clearance times than more fluid liposomes. The rigidity of a liposome can be enhanced by including cholesterol in the phospholipid bilayer. Increasing the rigidity of the lipid bilayer makes it harder for a lipoprotein to insert into the bilayer, an initial stage in the opsonisation of liposomes Small unilamellar vesicles (SUV), composed of distearoylphosphotidylcholine (DSPC) and cholesterol, were found to avoid RES detection to the greatest extent, but their small size tends to limit the amount of drug that may be encapsulated and thus their usefulness as drug delivery vesicles. Despite longer clearance times, these liposomes are still eventually cleared from the circulation by the RES.

In reviews of current trends in liposomal drug delivery Oku[111] and Allen[115] describe two techniques for increasing the circulation times of liposomes within the blood. Initial attempts at improving the circulation times of liposomes involved modifying the liposomal surface to mimic the natural surfaces of biomembranes, typically those of the erythrocytes. The sialic acid residues of glycoproteins and glycolipids are thought to play an important role in cell recognition and so liposomes have been modified with various gangliosides or sialic acid derivatives in order to fool the RES into thinking that such modified liposomes are not foreign. Monosialoganglioside (GM1)-liposomes have been shown to exhibit long circulation times[116,117]. Glucaronic acid has been investigated as a low cost alternative to sialic acid for the modification of liposomes.

The two are structurally similar, being biocompatible carbohydrates bearing carboxyl groups. Glucaronic acid can be incorporated into the liposome by chemical modification of the C-1 position of the sugar residue with a hexadecyl group to give palmatidylglucaronide. Potential problems with the commercial acceptability of liposomes containing GM1[115] arose because of its relatively low natural occurrence and difficulties in purification and synthesis[117]. These factors led to the GM1 type modified liposomes being superseded by PEG modified liposomes. In addition, naturally occurring glycolipids may become immunogenic upon inclusion into artificial membranes at concentrations higher than are normally encountered[117]. Surface modification with PEG derivatives utilises the steric stabilisation approach adopted with nanoparticles. Thus the rate of opsonisation of the liposomes is reduced by increasing the surface hydrophilicity of the liposome by modification with PEG derivatives. Liposomes containing PEG-phosphotidyl ethanolamine were found to have longer circulation times than unmodified liposomes due to reduced RES trapping[117].

2.3.3 Micellular drug delivery devices.

The main limitations of the steric stabilisation technique for both nanoparticles and liposomes are competitive desorption of the stabilising polymer and poor initial coverage of the surface which allows partial opsonisation. Rolland *et. al.*[118] prepared micelles and polymerised micelles based on poly (oxyethylene-b-isoprene-b-oxyethylene) in an attempt to prepare a more biocompatible surface. Thus micelles were prepared by dissolving 0.1% of the block copolymers in distilled water with stirring over 24hrs at temperatures between 20 and 70°C. The micellular solutions were then filtered through 0.45μm or 0.22μm filters and/or sonicated to reduce the polydispersisities of the micelles. The hydrophobic cores of the micelles were then photocrosslinked through residual double bonds on the isoprene moieties by exposure to UV light for 4hrs with a photoinitiator (azo-bis-*iso*-butyronitrile).

The crosslinked micelles were shown to be spherical, discrete and near monodisperse, ranging in size from 200nm to 120nm depending on the temperature of preparation (20°C and 70°C respectively). Crosslinking caused very little decrease in diameter of the particles. Crosslinking was also carried out in the presence of ^{14}C labelled styrene monomer. The labelled styrene was copolymerised within the hydrophobic core and used as an *in vitro* and *in vivo* assay to determine the bodily distribution of the particles in mice. Styrene homopolymer was found to form bridges between the nanoparticles. These were removed by extraction in petroleum ether 60/80 to form discrete microspheres with a mean diameter of 102nm and a polydispersity index of 0.125 (derived from the width of the particle size distribution). The particles were shown to reside almost completely in the blood after 2 hrs and approximately 80% of the administered dose was still present in the blood after 24hrs. Of the remaining 20%, 10% was localised in the spleen and 10% in bone. These values compared favourably with those obtained by Allen *et. al.*[115] for liposomes modified with GM1 gangliosides. These workers found approximately 80% of the administered dose in the blood stream after 2hrs with 10% in the liver and 10% in the bones and only 30% of the administered dose still present in the blood after 24hrs with approximately 30% in the liver and 40% in the bones. However, the liposomal system developed by Allen *et. al.* could conceivable be used as a drug delivery device in humans whereas the non degradable particulate system developed by Rolland *et. al.*[118] was used as a model system to study the steric stabilising effect of PEO polymers. True micellular micro vesicles for drug delivery have also been developed. Kwon *et. al.*[119] describe two techniques used to incorporate hydrophobic drugs into micelles. The drug can either be physically entrapped within the hydrophobic core of the micelle formed from an inherently amphiphilic block copolymer such as poly (ethylene oxide-b-propylene oxide-b-ethylene oxide)[120], poly (polyethylene oxide-b-DL lactide)[121] and poly (ethylene oxide-b-benzyl L-aspartate)[119] or chemically bound to the backbone of a block copolymer in which one of the blocks contains functional groups suitable for covalent linking of the hydrophobic drug moiety such as poly (ethylene oxide-b-aspartic acid)[119].

The resulting polymer-drug conjugate then exhibits amphiphilicity and may adopt a micellular conformation. This concept has been extended to include hydrophobic core formation through coordination of cisplatin by the carboxyl groups of the aspartic acid moieties of poly (ethylene oxide-b-aspartic acid) block copolymers[119]. In addition to showing prolonged circulation times and altered biodistribution because of RES avoidance, micellular vesicles offer additional advantages over liposomal and microparticulate delivery systems because of their decreased size. Extravasation of the micellular vesicles and their entrapped or bound drug through leaky endothelial walls is of particular importance. This is discussed in more detail in Sec. 2.4. The small size of micellular delivery devices makes intravenous injection a safe process Polyethylene oxide based block micelles have hydrodynamic diameters in the range 10-30nm and the micelles have been shown to consist of in the order of 10^2 unimers. Thus the micellular, as distinct from the unimer, molecular weight is well in excess of the renal threshold, preventing their excretion. The micelles are extremely stable relative to low molecular weight micelles which dissociate rapidly below their critical micelle concentration (typically milliseconds). Block copolymeric micelles dissociate slowly over a period of hours or days (as shown by GPC). Thus drug delivery mediated by micelle degradation can be extended over a period of hours to days[119]. The stability of the micelle can be altered by changing the ratio of hydrophilic to hydrophobic chain lengths or by altering the nature of the hydrophobic block. Trubetskoy *et. al.*[122] suggested that the two fatty acid acyls of lipids may confer micelles comprising of polyethylene oxide capped with lipids with increased stability by increasing the degree of interchain hydrophobic interactions within the core of the micelle. Thus micelles of diacyl lipid-polyethylene oxide conjugates were investigated as colloidal particles for perceutaneous lymphatic delivery vehicles. These workers proposed that increased micellular stability under physiological conditions could be achieved by utilising larger hydrophilic blocks with more lipophilic hydrophobic blocks. This was supported by the relatively higher stability of liposomes sterically stabilised with PEO capped with palmityl (C_{16}) fatty acid acyls compared to decyl (C_{10}) derivatives.

Kwon *et. al.*[119,123,124] found that polyethylene oxide-polyaspartic acid-doxorubicin conjugates showed greatest micellular stability with high molecular weight PEO blocks (5000-12000 $gmol^{-1}$) and low molecular weight polyaspartic acid-DOX blocks (2000 $gmol^{-1}$). Decreasing the molecular weight of the PEO blocks (1000$gmol^{-1}$) whilst increasing the polyaspartic acid-DOX resulted in the formation of unstable micelles which rapidly dissociated and were cleared through the kidneys. Increasing the molecular weight of the polyaspartic acid blocks for a given PEO molecular weight cause increased RES uptake indicating less effective shielding of the growing hydrophobic core by the PEO. Piskin *et. al.*[121] developed novel block copolymer micelle forming polymers from poly (ethylene oxide-b-DL lactide) by transesterification of poly (DL-lactic acid) (Mn 1886 ± 100) with PEG (Mn 3300-4000). Drug delivery of physically entrapped drugs was envisaged to proceed through diffusion or through degradation of the poly (DL lactide) blocks. Micelles were prepared with PEG contents of 25-80%. These workers found that PEG contents of 25% produced small compact micelles due to the tightly packed nature of the hydrophobic poly (DL Lactide) blocks in copolymers with a low degree of hydrophilicity. As the percentage of PEG was increased, the molecular weight of the micelles increased to a maximum at 35% and then decreased again. The maximum average diameter of the micelles was obtained with a PEG content of 60%. Increasing the ratio of the hydrophilic segments was considered to result in the formation of larger but more loosely packed micelles. At a PEG content of 80%, the resulting micelles were extremely unstable. Maximum drug loading (approximately 21mg of adriamycin. HCl/g of copolymer) was obtained with the micelles of highest molecular weight at 35% PEG content. At lower PEG contents the increasingly compact hydrophobic cores reduced the available space for accommodating the drug, whilst at higher PEG contents the increased hydrophilicity of the micelles resulted in a more loosely packed core which exhibited a lower compatibility with the adriamycin. HCl. The micelles showed extended release of adriamycin. HCl *in vitro* over a period of 5 weeks. The relatively slow release was attributed to a degradation controlled release pattern.

Once the poly (DL-lactic acid) blocks degrade below a certain size, the copolymer becomes soluble and the macromolecular conformation is disrupted causing release of the entrapped drug. The intact micellular structure provides an effective barrier to diffusion of the drug. Kwon *et. al.*[119] found that residual doxorubicin physically entrapped in poly (ethylene oxide)-b-poly (aspartic acid -doxorubicin) was released slowly over a period of five days. In this case the slow release of unbound doxorubicin was attributed to diffusion of the unbound drug from the micellular drug polymer conjugate. This offers additional control over drug delivery. An initial relatively high dose could be administered through diffusion followed by a controlled release via degradation over a period of weeks.

2.3.4 Hypercoiling polymers as potential drug delivery vehicles.

Hypercoiling polymers are weak polyanions or polycations which also contain asymmetrically positioned hydrophobic moieties within, or as pendant groups on, a flexible backbone. These polymers exist in an extended conformation when the majority of their ionisable groups are in a charged state, but form tightly coiled, highly compact, conformations stabilised by hydrophobic bonding on loss of that charge, eventually precipitating from solution. At intermediate degrees of ionisation, the polymers exhibit amphiphilic properties and exist as unimolecular micelles. Tonge *et. al.*[125] have recently shown that this phenomena is dependent on the molecular weight of the polymer and below a critical molecular weight, no amphiphilic properties are seen. Tonge[126] has proposed the use of such polymers as novel delivery devices capable of responding to local pH stimuli. Thus hypercoiling polymers in the amphiphilic state could be used to encapsulate hydrophobic drugs within the core of the amphiphile. Selective delivery would be achieved by expansion of the polymer to the extended conformation in response to localised pH change. The relatively acidic microenvironment of some solid tumours presents an ideal opportunity for targeting by such a mechanism. Manipulation of the hydrophobic/hydrophilic balance within the polymer gives control over the pH range of conformational transition.

The switch in conformation occurs over a narrow enough pH range to make such a mechanism feasible, though whether the specific pH range at which the conformation change occurs can be manipulated to the pH of the tumour microenvironment, through structural modification of the polymer, remains to be seen. Hypercoiling polymers form unimeric amphiphiles and so may be small enough to avoid RES detection in a similar manner to soluble macromolecules. Several non degradable hypercoiling polymers are known, but as far as this author is aware no biocompatible, biodegradable hypercoiling polymers exist. Possible synthetic routes to biodegradable hypercoiling polymers and structural manipulation of potential hypercoiling polymers have been investigated in these laboratories. Biodegradability is important since amphiphilic properties of potentially hypercoiling polymers may only become evident at molecular weights above the renal threshold. For application as drug delivery vehicles biodegradability would be required to prevent accumulation of the carrier in the host.

2.4 TARGETED DRUG DELIVERY.

Targeting of drugs, polymer-drug conjugates and drug delivery devices such as colloidal carriers to sites of required activity can be subdivided into two categories, i.e. active and passive targeting. Active targeting involves modification of the drug with a targeting moiety such as a monoclonal antibody or antibody fragment specific for a particular cell type and will not be considered in detail. The uptake of colloidal carriers by the RES is an example of passive targeting and may be exploited in the treatment of diseases that involve the RES such as macrophage neoplasms, enzyme deficiencies and associated storage diseases[108]. If this can be prevented then it is possible to redirect the colloidal carrier with their payload to alternative sites. Passive targeting takes advantage of specific physiological abnormalities of the diseased tissue. Perhaps the most important example of passive targeting is the selective accumulation of macromolecules and colloidal carriers within the extra cellular fluid of solid tumours. This effect is termed enhanced permeability and retention (EPR)[42] and can be used to selectively deliver colloids and their therapeutic payloads directly to the site of a solid tumour. The colloidal carriers can then provide a controlled supply of the therapeutic (or diagnostic) agent to the diseased tissue.

Targeted delivery offers major clinical advantages over systemic drug delivery. The direct delivery of the drug to the site where it is needed reduces the total amount of drug required and precludes possible side effects arising because of systemic exposure of undamaged tissue to the effects of the drug. This problem is especially serious in the treatment of cancers because of the low therapeutic ratio often exhibited by the chemotherapeutic agents used. The maximum dosage of free doxorubicin for example is limited by its cardiotoxicity. Administration in liposomal or micellular form allows much higher dosing since the drug is delivered selectively to the site of the tumor[115,119-124]. As its name suggests, EPR consists of two separate phenomena which are specific to solid tumours. The first phenomena is the often enhanced permeability of solid tumour vasculature[108,42]. Most non specialised vascular tissue consists of continuous endothelial cells with tight cell junctions (< 2nm) and a continuous basement membrane. Post capillary venuoles may have slightly larger cell junctions (<6nm). These tissues are, therefore, impermeable to particles of colloidal dimensions (typically 20-50nm). Certain specialised tissues exhibit a discontinuous endothelium. Thus, the kidney glomerulus and the pancreas have fenestrated intercell junctions (40-60nm) and could allow passage of colloidal vesicles. Certain tumours also exhibit fenestrated cell junctions. A continuous basement membrane is normally present in these tissues which prevents access of colloidal particles to the interstitial sites. The liver, spleen and red bone marrow have sinusoidal cell junctions with the largest intercellular spaces (<150nm). Blood vessels of the liver have no basement membrane and in the spleen and bone marrow, the membrane is discontinuous[42]. Certain tumours also exhibit this type of blood vessel architecture. Inflamed and infected tissue may contain blood vessels with disrupted endothelium and basement membranes[108,42]. These blood vessels are particularly permeable to colloidal and macromolecular species.

The second phenomena is the limited lymphatic drainage exhibited by some tumors[42,127]. In most tissue, molecules in the interstitial fluid are returned to the bloodstream either directly through the enlarged cell junctions of the postcapillary interendothelial cell pores or via the lymphatic drainage system. The lymphatic system is a one way drainage system which allows passage of molecules (and macromolecules) through enlarged interendothelial cell junctions at the end situated in the interstitium. The lymphatic fluid then drains back to the venous system via a series of vessels and nodes. Larger macromolecules tend to rejoin the circulation via the lymphatic system whereas smaller macromolecules can rejoin more easily through the post capillary venuoles. Within solid tumours, the lack of an effective drainage system means that macromolecules must rejoin the circulation via the postcapillary venuoles or diffuse through the tumour interstitium until they reach lymphatic vessels in adjacent normal tissue. Macromolecules too large to be drained via the postcapillary venuoles are retained within the tumour interstitium. In addition to its relevance to colloidal vesicles, the EPR effect also applies to soluble macromolecules. The concept of utilising a soluble macromolecular carrier for drug delivery has long been recognised. The main advantage of this method of drug delivery is the potential tractability of such systems[42-130]. In the mid 1970s, Ringsdorf[131] presented a model for pharmacological polymers that allowed modification of solubility, toxicity, targeting and activity of the conjugated drug. Hoffman[132] has extended this model, specifying specific biologically active species which may be attached to the polymeric carrier in order to confer the polymer conjugate with biological activity. Such groups include pharmacons, signal groups for imaging, lipophilic groups for insertion into cell membranes and steric stabilising groups. The main advantage of using polymeric carriers for drugs is the relatively high molecular weight of the carrier compared to the drug and the multifunctionality available with macromolecules. Thus the physiochemical properties of the drug conjugate can be varied from those approximating the polymer carrier at low degrees of substitution to novel characteristics such as micellisation at higher degrees of substitution.

The drug may be attached or included in an active form or may be substantially less active, regaining its activity upon hydrolytic or enzymatic degradation of a connecting spacer arm after pinocytotic uptake of the polymer-drug conjugate into the intracellular lysozomal compartments. Once in the lysozomal compartment enzymatically or pH sensitive peptide spacer groups cause release of the covalently attached drug. The polymer drug conjugate may then be considered as a pro-drug. Systemic side effects can be reduced and specific intratumoural accumulation enhanced so as to result in a higher therapeutic ratio for the drug-polymer conjugate system compared to the free drug. Duncan[127] has recently reviewed the use of soluble drug-polymer conjugates in the field of cancer chemotherapy outlining the specific characteristics required of a macromolecular drug carrier system. One of the main pre-requisites of the macromolecular carrier is biocompatibility. Polymer related toxicity is generally disadvantageous, but may be useful if the effects could be targeted. Encouraging *in-vitro* and *in-vivo* results have been obtained using dextran, N-(2-hydroxypropyl)methacrylamide and polyamino acids such as polyaspartic acid as inert carriers for chemotherapeutic agents such as doxorubicin and mitomicin[42,129,130]. The synthetic vinyl polymers such as N-(2-hydroxypropyl)methacrylamide are non biodegradable and so are limited to molecular weights below the renal threshold. Since maximum retention and specific uptake of the macromolecule by a solid tumour occurs for molecular weights above the renal threshold, this requirement is an obvious disadvantage[128]. Natural degradable polymers of higher molecular weights can be used, although covalent conjugation of pendant groups may reduce susceptibility to enzymatic attack with a consequent reduction in the biodegradability. Immunogenic response to degradation products may also limit the applicability of degradable polymers[127]. Two soluble polymeric drug carriers are currently in clinical use. The first, SMANCS[129], is a polymer protein conjugate consisting of two molecules of poly (styrene-co-maleic acid/anhydride) covalently linked via amide linkages to the proteinaceous anti-tumour agent neocarzinostatin. The conjugate has a 10-fold higher plasma half life than the free protein, causes macrophage activation, stimulates interferon activity and is generally immunostimulatory[127].

The conjugate is administered intraarterially in lipiodol, a lymphographic agent showing high liver selectivity, followed by gelfoam powder embolisation and has given impressive results in the treatment of non resectable hepatocarecinoma[42,127]. This method of delivery is an example of chemoembolisation and is discussed in more detail in Sec. 2.5. Tumour to blood ratios of 2500 to 1 have been observed in rabbit liver models[127]. No other tumour targeted delivery technique has afforded such a high ratio. Aqueous solutions of SMANCS have proved to be effective against various solid tumours (lung, stomach, ovary, adrenal, brain and esophageal) but not against metastatic melanoma or metastatic bone tumors[127]. The second soluble polymeric drug carrier system make use of monomethoxy PEG-protein conjugates and are where most clinical investigations have been reported. Conjugation increases solubility, plasma half-time and reduces immunogenicity of the protein[127]. One of the main drawbacks to conjugation of proteins is the decreased biological activity in the resultant conjugate, although the increased plasma circulation time may compensate for this[127]. Monomethoxy-PEG asparaginase is currently undergoing clinical trials as a cancer chemotherapeutic agent in the treatment of lymphoblastic leukemia and lymphomas[127].

2.5 CHEMOEMBOLISATION

The primary metastatic site for many malignant tumours is the liver[133] which together with primary liver tumors[134] account for over one million deaths world-wide annually[135]. There are currently only two curative surgical treatments available, surgical resection and transplantation[136]. Estimates vary on the viability of surgery from less than 10%[137] to an upper limit of 30%[138]. Transplantation is expensive and, even if successful leaves the patient dependent on immunosuppresants[136]. The seemingly low percentage of operable cases is due to the link between primary HCC and hepatic cirrhosis[139] together with invasion of the disease into the surrounding tissue. Resection becomes increasingly difficult since the patient is either left to recover from a major surgical procedure with a diseased and potentially disfunctional liver[136] or has only a part of the viable tumour removed. Earlier diagnosis due to better screening techniques[136] and adjuvant therapy to simplify[140] the surgery and /or prevent recurrence is improving the success of these treatments.

Since existing curative techniques are limited by such underlying complications there has been considerable effort in the past fifteen years or so to find alternate methods to treat unresectable HCC[141]. This has lead to a variety of palliative treatments including perceutaneous ethanol injection and hormone therapy as well as more traditional chemo and radiotherapy which have proved particularly ineffective for HCC. More promising results are being obtained from modifications of these techniques including intraarterial infusion chemotherapy and increasingly chemoembolisation[142]. Chemoembolisation in its most simple form involves intra-arterial infusion of a chemotherapeutic agent to the effected area followed by the embolisation of the main arteries or arterioles supplying that area with blood. This results in a prolonged and regionalised cytotoxic effect from the anti cancer drug complemented by the necrosing effect of the embolisation process[143]. This procedure was developed to improve early results obtained from simple embolisation. It was found that the emulsification of a chemotherapeutic with iodised poppy seed oil not only allowed for an increased residence time, but resulted in preferential targeting of the tumour by the oily emulsion[144]. Embolisation using sterile gelatin foam further increases the residence time of the drug and reduces systemic exposure[145]. The technique has been extended further by the use of a variety of microspheres either as embolants or in a combined role as embolant and delivery system[146]. The technique has provided encouraging results giving relief of both mass and hormonal symptoms in many cases[147]. It is often used pre-operatively to reduce the size of a tumour[140] and post operatively to prevent recurrence[148]. In many cases after resection complete necrosis of a primary node is seen.

The main draw backs of the procedure are unwanted embolisation in unaffected areas especially if underlying liver disease is a problem[149], incomplete embolisation of a malignant area allowing leaching away of any drug and reducing any necrosing effect[150], neovascularisation[151] and the inability of the process to target daughter nodules in more remote sites of the liver. Occurrence of daughter nodules is possibly the main reason for the failure of this technique[152]. The problem lies in the fact that the embolant is delivered selectively to an effected area transarterially via a catheter. If the secondary site is remote from the area of administration it will not be effected.

Improvement in imaging techniques, in addition to providing earlier diagnosis of primary tumours, will allow improved location of daughter nodules[153] and their subsequent treatment.

One of the principal difficulties in treating cancers is the lack of consistent differences between malignant and normal tissue. Many of the current chemotherapeutic approaches to cancer treatment rely on the relatively high metabolic rate of malignant cells to differentiate them from normal tissue. That this approach is not reliable is easily demonstrated by the side effects commonly experienced by patients undergoing chemotherapy. Normal cells such as in the hair follicles and bone marrow also have high metabolic rates and are susceptible to the effects of certain chemotherapeutics.

The pH of the microenvironment surrounding a tumour cell is characteristically lower than in normal tissue. This is due to the relatively higher metabolic rate of the tumour coupled with the typically poor blood supply to neoplastic tissue[154]. These factors result in the formation of lactate which diffuses from the interstitial sites into the bloodstream and is metabolised by healthy liver. Hydrolysis of ATP in an energy deficient environment also contributes to the acidic microenvironment of the tumor[155,156]. It may be possible to use the pH difference between malignant and normal tissue to cause precipitation of macromolecules that are soluble at normal physiological pH, but insoluble at the pH of the microenvironment of the tumour. Two conditions are required for therapeutic efficacy. The polymer must be transported via the regional blood flow to the neoplastic site where it undergoes a pH driven chemical change leading to aggregation (or local release of covalently bound cytotoxic agent). For this to occur, the pH microenvironment at the site must be sufficiently low to favour the thermodynamically driven chemical change, whilst the reaction kinetics must be sufficiently rapid that the polymer aggregates at the site rather than elsewhere in the body.

The physiological conditions in regional blood capillaries are unlikely to satisfy these conditions as the buffering capacity of serum proteins and the relatively short blood residence time would counter the required pH drop. However, within the tumour interstitium, accumulation of the soluble macromolecule could occur by the EPR effect. The typically high interstitial pressure within solid tumours reduces fluid convection and molecular (proton and polymer) transport is primarily by diffusion.

If we assume that the pH profile and the soluble polymer distribution within the interstitium adjacent to blood capillaries can be approximated by a simple "diffusion-reaction model" we can envisage the following situation: In tumour interstitium high tumour cell metabolism leads to the generation of protons which diffuse from the site of production towards the capillary. In order to generate the concentration gradient for proton diffusion the pH drops radially from the blood capillary. Simultaneously, polymer is transported across the capillary endothelium and then diffuses radially outwards into the interstitium. At some point the pH is sufficiently low that localised polymer change occurs leading to the formation of a front of aggregated polymer. The specific point at which this occurs would be dependent upon the metabolic rate of proton generation, the permeability of the tumour endothelium and interstitium to polymer molecules and the pH dependent solubility characteristics of the polymer. In such areas of low local pH precipitation would retard diffusion of lactate away from the neoplastic site. Lactate accumulation is known to result in severe tumour acidosis[156]. The lowering of extracellular pH increases sensitivity of a cell to thermo[157], chemo[154] and radiotherapy[158] and may cause cell death by acidosis. This offers one main advantage over conventional embolisation therapy in that complete occlusion of an arteriole is avoided reducing damage to healthy tissue. The pseudoproteins can also be radio labelled and may provide a technique for site specific imaging.

3. CHAPTER 3

SOLUTION POLYCONDENSATION USING MISCIBLE MIXED SOLVENT

3.1 MATERIALS

Acetone (dried over calcium hydride, distilled and stored over 4a molecular sieve)	BP Chemicals
Benzene (99.8%) anhydrous	Aldrich
1,3-benzene di-sulphonyl chloride (97%)	Aldrich
Carbon tetra chloride tech. grade	BDH
Chloroform (99.9%) A.C.S. HPLC grade (stabilised with amylenes)	Aldrich
18-crown-6	Sigma
L-2,4-Diamino Butyric acid (98%)	Aldrich
DL-2,3-Diamino propionic acid monohydrochloride (98%)	Aldrich
Meso-2,3-Diamino succinic acid (90%)	Sigma
Dichloromethane(99.9%) A.C.S. HPLC grade (stabilised with 50-150ppm hydrocarbons)	Aldrich
Diethyl malonyl chloride (98%)	Aldrich
Dimethylformamide (99%). (GPR grade)	Fisher
Dimethylsulphoxide	BDH
Ethyl malonic acid (97%)	Aldrich
Iso phthaloyl chloride (98%)	Aldrich
Itaconyl chloride (90%) tech. grade	Aldrich
L-Lysine free base (97%)	Aldrich
L-Lysine ethyl ester dihydrochloride (>99%)	Fluka
L-Lysine monohydrochloride (99 +%)	Aldrich
L-Ornithine monohydrochloride (99 %)	Aldrich
Methyl cellulose (Mn 14,000)	Aldrich
3-Phenyl glutaric acid (97%)	Aldrich
Phenyl malonic acid (98%)	Aldrich
Phenyl succinic acid (98%)	Aldrich
Polycaprolactone diol (Mn ca. 2000)	Aldrich

Poly ethylene glycol (1000,2000 and 3400)	Aldrich
Polyvinyl alcohol 98% hydrolysed (Mn 13,000-23000)	BDH
Potassium carbonate (99%) A.C.S reagent grade	Aldrich
Potassium chloride (99%) A.C.S reagent grade	Aldrich
Potassium hydroxide (85%) A.C.S reagent grade	Aldrich
Tetra butyl ammonium bromide (98+%) A.C.S reagent grade	Aldrich
Tetrahydrofuran(99.9%) HPLC grade (inhibitor free)	Aldrich
Toluene (99.5%) A.C.S. grade	Aldrich
Sodium carbonate (99.5%) A.C.S reagent grade	Aldrich
Sodium chloride (99%) A.C.S reagent grade	Aldrich
Sodium hydroxide(97 + %) reagent grade	Aldrich
Sodium oleate (98%)	Aldrich

3.2 POLYCONDENSATION OF DIAMINES AND DIACYL CHLORIDES USING MISCIBLE SOLVENTS.

Polycondensation in miscible aqueous/organic systems was investigated as a method for synthesising polyamides and copolyamides based on diamino acids and their esterified derivatives with diacyl chlorides. If the organic solution of diacylchloride is miscible with the aqueous solution of acid acceptor and diamine then the increased contact between reagents may improve the yield molecular weight of the resulting polymer. On the other hand the diacyl chloride is also more likely to undergo hydrolysis leading to chain termination and a subsequent reduction in molecular weight. Reaction in miscible solvents is likely to be useful in situations where the acid chloride shows a considerable resistance to hydrolysis and where contact between reagents is a problem in a two phase system such as when the diamine contains additional hydrophilic functional groups. Aromatic diacyl chlorides are relatively resistant to hydrolysis whereas the highly reactive aliphatic diacyl chlorides are readily hydrolysed and are, therefore, less likely to give high molecular weight polymer in a miscible organic/aqueous system. Changing to a single phase reaction system is likely to change the reaction kinetics from the diffusion controlled kinetics of a genuine interfacial reaction to a kinetically controlled system seen in solution polymerisations.

3.3 GENERAL METHOD FOR POLYCONDENSATION IN MISCIBLE SOLVENT SYSTEMS.

Reagent grade acetone was dried over calcium hydride and distilled before use. The dried acetone was kept over 4a molecular sieve. In a typical reaction, 50mL of a 0.2M aqueous solution of lysine ethyl ester. 2HCl with the required amount of acid acceptor was stirred with a magnetic stirrer (unless otherwise specified) or an overhead stirrer in a 250mL beaker with four diametrically opposing one cm integral baffles protruding into the vessel. To this was added rapidly 50mL of 0.2M diacyl chloride solution in dry acetone.

In cases where the polymer was precipitated during the reaction, coagulation generally occurred to give a solvent-swollen mass and the polymer was easily removed with a spatula. Filtration of the acetone water solution did not generally yield any more precipitate. When the resulting polymer contained pendant carboxylate groups, the reaction medium was acidified to effect precipitation. As in the previous case, coagulation facilitated isolation of the polymer with a spatula. Any product was washed with acetone and water, then dried overnight in a vacuum oven at 55°C. The precipitated polymers were generally swollen by the organic solvent and expanded several fold on drying.

3.4 POLYCONDENSATIONS BASED ON *ISO*-PHTHALOYL CHLORIDE AND LYSINE ETHYL ESTER.

3.4.1 Polycondensation of *iso*-phthaloyl chloride and lysine ethyl ester. 2HCl using miscible acetone/aqueous potassium carbonate systems.

50mL of 0.2M *iso*-phthaloyl chloride were reacted with 50mL of 0.2M lysine ethyl ester. 2HCl/0.5M aqueous potassium carbonate solution for 1 hr according to the general method (sample code ME 3.1).

An initial vigorous effervescence which subsided after a few seconds was noted. A cream coloured gummy ball of precipitate formed during the course of the reaction leaving an essentially clear solution. The solution was filtered under vacuum and the precipitate washed with distilled water (yield 74%). On washing the surface of the creamy gummy precipitate became white and crumbly. The filtrate became cloudy on the addition of water.

FT-IR analysis showed strong absorptions at 1736cm^{-1} (ester C=O stretch), 1642cm^{-1} (amide band I) and1535cm^{-1} (amide band II).

3.4.2 Effect of concentration of acid acceptor on polycondensation of lysine ethyl ester. 2HCl and *iso*-phthaloyl chloride using miscible acetone/aqueous potassium carbonate systems.

50mL of 0.2M *iso*-phthaloyl chloride were reacted with 50mL of 0.2M aqueous lysine ethyl ester solution containing potassium carbonate solution for 1 hr according to the general method. The concentration of the acid acceptor was increased by 0.05M steps over the range 0.50M to 0.85M (sample codes ME 3.2.1-ME 3.2.8).

Initial effervescence was seen with acid acceptor concentrations of 0.55M, 0.6M and 0.65M. No effervescence was seen with increasing concentrations of acid acceptor. In these cases, the single resultant phase became opaque on mixing of the two phases eventually yielding a creamy precipitate. A precipitate was produced at the liquid/air interface overnight. The precipitates produced were analysed by FT-IR (Appendix B. Table B1. Spectrum 1) and all showed strong absorptions at 1735cm^{-1} (ester C=O stretch), at 1642cm^{-1} (amide band I) and 1535cm^{-1} (amide band II.) The spectrum of the film that formed overnight also showed these bands indicating that this product was structurally similar to the initial precipitate and not hydrolysed acid chloride. It was assumed that this was oligomeric, low molecular weight material that remained soluble in the acetone/water system, but precipitated on evaporation of the acetone overnight. The low molecular weight of the material forming such films was confirmed by GPC (Sec 5.4.2 Table 5.7)

Addition of water to the filtrate during the washing process caused clouding of the filtrate. The filtrate cleared again on addition of acetone.

3.4.3 Effect of reaction time on polycondensation of lysine ethyl ester. 2HCl and *iso*-phthaloyl chloride using miscible acetone/aqueous potassium carbonate systems.

50mL of 0.2M *iso*-phthaloyl chloride/acetone solution was added rapidly to 50mL of 0.2M lysine ethyl ester. 2HCl/0.6M potassium carbonate (4.1463g) aqueous solution and stirred for 30 minutes (sample code ME 3.3.1). A creamy precipitate was isolated by filtration and dried overnight in a vacuum oven (yield 75.9%).

The reaction was repeated for 60 and 90 minutes (Sample codes ME 3.3.2-ME 3.3.3). In both cases creamy precipitates were produced (yield 76.4% and 81.6% respectively). The FT-IR spectra of the precipitates (Appendix B. Table B1. Spectrum 1) showed strong absorptions at 1737cm^{-1} (ester C=O stretch), 1642cm^{-1} (amide band I) and at 1535cm^{-1} (amide band II).

3.4.4 Effect of added potassium chloride on polycondensation of lysine ethyl ester. 2HCl and *iso*-phthaloyl chloride using miscible acetone/aqueous potassium carbonate systems.

Added potassium chloride reduces the miscibility of the two phases and may result in a salting out effect on the diamine or any low molecular weight oligomers in solution decreasing the average molecular weight and increasing the polydispersity.

50mL of 0.2M *iso*-phthaloyl chloride/acetone solution was added rapidly to 50mL of 0.2M lysine ethyl ester. 2HCl/0.6M potassium carbonate (4.1463g) aqueous solution containing 1,2 or 3g of potassium chloride (sample codes ME 3.4.1-ME 3.4.3).

Initial vigorous effervescence was followed by precipitation of the polymer. The yield dropped from 75% with 1g of added potassium chloride to 68% with 2g and 62% with 3g. This was believed to be due salting out of the polymer at lower molecular weight.

The FT-IR spectra of the products from the reaction and the overnight precipitate (Appendix B. Table B1. Spectrum 1) all showed absorptions at 1736cm^{-1} (ester C=O stretch),at 1637cm^{-1} (amide band I) and at 1535cm^{-1} amide band II).

3.4.5 Effect of organic solvent on polycondensation of lysine ethyl ester. 2HCl and *iso*-phthaloyl chloride using miscible solvent systems.

50mL of 0.2M *iso*-phthaloyl chloride in tetrahydrofuran was added rapidly to 50mL of 0.2M lysine ethyl ester. 2HCl/0.6M potassium carbonate (4.1463g) aqueous solution and stirred for 60 minutes. The initial vigorous effervescence seen in comparable acetone systems was not observed and precipitation was not as rapid (sample code ME 3.5).

A creamy precipitate was isolated by filtration with further precipitation occurring in the filtrate overnight (combined yield 98%).

The FT-IR spectrum of the initial product from the single phase reaction and the overnight precipitate (Appendix B. Table B1) both showed strong absorptions at 1736cm^{-1} (ester C=O stretch) at 1642cm^{-1} (amide band I) and at 1535cm^{-1} (amide band II).

3.4.6 Effect of stirring and scale up on polycondensation of lysine ethyl ester. 2HCl and *iso*-phthaloyl chloride using miscible solvent systems.

Samples of poly (lysine ethyl ester *iso*-phthalamide) were prepared in a 2 L resin flask. 500mL of 0.2M lysine ethyl ester. 2HCl/0.8M potassium carbonate solution were stirred at 2000 r.p.m. 500mL of 0.2M *iso*-phthaloyl chloride solution in acetone were added rapidly. Stirring was continued for 2 hrs (sample code ME 3.6.1.1). A large solvent-swollen ball of polymer was recovered from the blades of the stirrer (yield 72.3%). Scaling down by a factor of 1/2 resulted in an increase in yield to 84%

The FT-IR spectra of the precipitates (Appendix B. Table B1. Spectrum 1) showed strong absorptions 1737 cm^{-1} (ester C=O stretch), at 1637cm^{-1} (amide band I) and 1535 cm^{-1} (amide band II).

The 1 L scale experiment was repeated using 0.6M potassium carbonate and an almost instantaneous vigorous effervescence occurred causing the level of liquid to rise to the top of the resin flask This was accompanied by the rapid precipitation of polymer (sample code ME 3.6.2). The rate of stirring was reduced to 200 r.p.m. and the precipitated polymer isolated after 30 minutes, washed with distilled water and dried overnight in a vacuum oven at 55°C (yield 65%).

In all cases a thin film formed at the liquid air interface overnight (sample code ME 3.6.1.2).

Variation in yields of product vin systems where the only change was the scale of the reaction were attributed to variations in stirring efficiency. A higher yield was obtained in the baffled 1 L resin flask compared to the 2 L unbaffled resin flask. One would expect the stirring efficiency to be lower in the second case in the absence of baffles and with a larger ratio of reactor to impeller diameter. Similar drops in yield were noted on decreasing the size of magnetic stirrer. Decreasing the length of the magnetic stirrer from 38mm to 25mm caused a drop in yield from 76.4% to 70% in reactions that were otherwise identical.

3.4.7 Effect of time of addition of second phase on polycondensation of lysine ethyl ester. 2HCl and *iso*-phthaloyl chloride using miscible acetone/aqueous potassium carbonate systems.

The time of addition of the second phase and the order of phase addition was varied and its effect on molecular weight distribution investigated.

50mL of 0.2M *iso*-phthaloyl chloride/acetone solution and 50mL of 0.2M lysine ethyl ester. 2HCl/0.6M potassium carbonate (4.1463g) aqueous were prepared according to the general method. Diacyl chloride was then added rapidly to diamine as in the previous experiment (sample code ME 3.7.1). The solution was stirred with a magnetic stirrer.

The experiment was then repeated, with the addition of the diacyl chloride using a 50mL burette to provide slow, controlled release into the diamine solution (sample code ME 3.7.3). The order of addition was then reversed, i.e. rapid addition of diamine solution to diacyl chloride solution (sample code ME 3.7.2) followed by slow diamine addition to diacyl chloride solution (3.7.4.1).

When either phase was rapidly added to the other or when the diacyl chloride was added slowly to the diamine then a creamy white precipitate was obtained (yield approximately 71%). When the diamine was added slowly to the diacyl chloride the yield was only 26%.

FT-IR spectra of all the products showed absorptions at 1737cm^{-1} (ester C=O stretch), at 1637cm^{-1} (amide band I) and 1535cm^{-1} (amide band II). An additional peak at 1790cm^{-1} was present in the FT-IR spectrum of the reaction where the diamine was added slowly to the diacyl chloride and the ester C=O peak shifted slightly to 1730cm^{-1} (Appendix B. Table B2. Spectrum 2). Initially this was assigned to the C=O stretch of an imide formed by reaction of the diacyl chloride with the secondary amides in the backbone of the growing polymer. A peak in this region of the infra red spectrum and a second at around 1710cm^{-1} is indicative of the presence of a five membered imide ring [44,45,127]. These band assignments, however, apply to cyclic imides and occur at roughly 30cm^{-1} higher in the spectrum of typical linear imides. Imide formation in the interfacial synthesis of polyamides is known to occur under certain conditions and is discussed in Sec. 1.4.3.3. The 1,3-substitution pattern of *iso*-phthaloyl chloride precludes formation of 5 and 6 membered imide rings. Hydrolysis of the ester group and subsequent reaction of the carboxyl group could only lead to 4 or 7 membered imide ring formation and so is also unlikely. The peak at 1790cm^{-1} is in the region of the spectrum associated with C=O stretching of an aromatic anhydride.

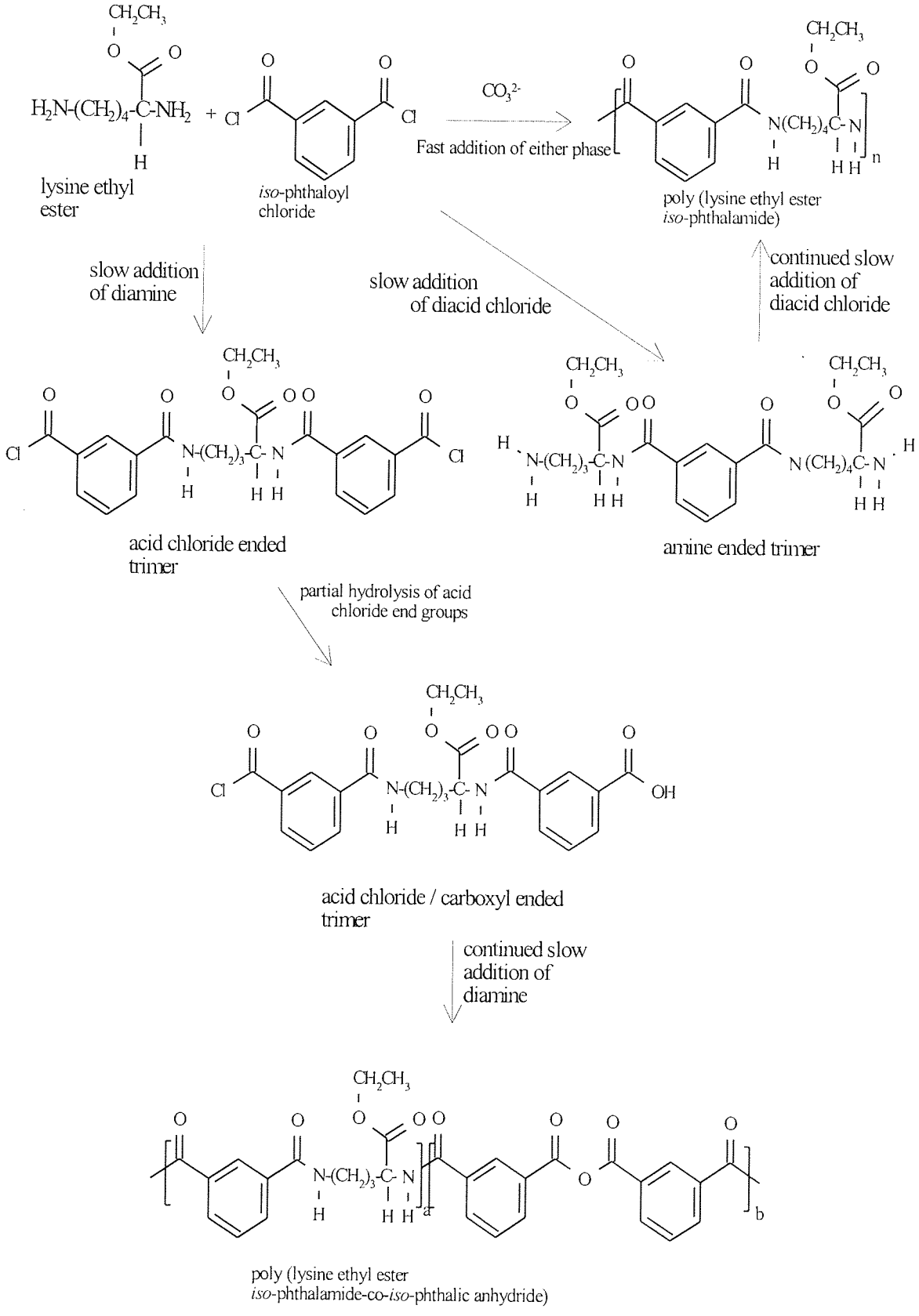
Anhydride formation is a potential competing reaction when lysine is used as the diamine because of potential reaction of the acyl chloride with the α-carboxyl group, but esterifying the carboxyl group would be expected to protect against this possibility. Hydrolysis of the ester does occur rapidly in 1.6M NaOH (Sec. 4.5.1), but not to any significant degree in potassium carbonate solutions and so the carboxyl groups must originate from hydrolysed acyl chloride (Fig. 3.1). In the initial stages of the reaction, diacyl chloride is present in a large excess and so any amine functions will be acylated to give acyl terminated oligomers. Partial hydrolysis of these oligomers would result in carboxyl terminated oligomers which may then be acylated by the diacyl chloride or acyl terminated oligomers which would still be present in an excess.

A second peak due to the C=O asymmetric stretch is normally seen in anhydrides. In benzoic anhydride this peak is at 1722cm^{-1} and so may be masked by the peak at 1730cm^{-1} due to the C=O stretch of the ester. Overlap of the two peaks would explain the slight shift in the position of the absorption from 1737cm^{-1} to 1730cm^{-1}. Hydrolysis of the diacyl chloride was also suggested by the marked reduction in the molecular weight of the resulting polymer (Sec 5.4.2)

3.4.7.1 Cleavage of anhydride with concentrated sulphuric acid.

The product from the previous reaction was dissolved in concentrated sulphuric acid and then reprecipitated in an excess of cold water (0°C). The absorption band at 1789 cm^{-1} in the FT-IR spectrum (Appendix B. Table B2. Spectrum 2) disappeared whilst the rest of the spectrum remains unchanged indicating complete cleavage of the anhydride linkages (sample code ME 3.7.4.2).

Fig. 3.1 Formation of in chain anhydride linkages with a limited supply of diamine in the miscible mixed solvent method.



3.4.8 Polycondensation of *iso*-phthaloyl chloride and lysine methyl ester using miscible acetone/aqueous potassium carbonate systems.

50mL of 0.2M lysine methyl ester. HCl/0.5M aqueous potassium carbonate solution was reacted with 0.2M *iso*-phthaloyl chloride/acetone solution for 1 hr according to the general method (sample code ME 3.8.1). The reaction was repeated with fast addition of the aqueous phase to the organic phase (sample code ME 3.8.2) and slow addition of the aqueous phase to the organic phase (sample code ME 3.8.3).

In either case where one phase was added rapidly to the other, an initial vigorous effervescence occurred, followed by the precipitation and coagulation of a white gummy material (yield 73% and 75%). In the case of slow addition of the aqueous phase to the organic phase only slight effervescence was seen with no rapid precipitation. A precipitate began to form after approximately 15 minutes (yield 20%).

The FT-IR spectrum (Appendix B. Table B2. Spectrum 3) of the precipitate produced on rapid addition of the second phase showed strong absorptions at 1736cm^{-1} (ester C=O stretch), at 1637cm^{-1} (amide band I) and at 1535cm^{-1} (amide band II). A further peak at 1790cm^{-1} (anhydride C=O sym. stretch) was present in the FT-IR spectrum (Appendix B. Table B2. Spectrum 3) of the precipitate produced on slow addition of the aqueous phase to the organic phase. The appearance of this peak has been discussed in Sec. 3.3.7.

3.5 POLYCONDENSATIONS BASED ON *ISO*-PHTHALOYL CHLORIDE AND NON ESTERIFIED DIAMINES.

3.5.1 Polycondensation of *iso*-phthaloyl chloride and lysine using miscible solvents.

50mL of 0.2M *iso*-phthaloyl chloride were reacted with 50mL of 0.2M lysine HCl/0.5M aqueous potassium carbonate solution according to the general method (sample code ME 3.9).

Stirring was continued for 45 minutes and then the acetone was removed by rotary evaporation to leave a straw coloured solution. Acidification with concentrated hydrochloric acid gave a white precipitate (yield 87%).

The FT-IR spectrum (Appendix B. Table B3. Spectrum 4). showed strong absorptions at 1707 cm^{-1} (carboxylic acid C=O stretch), at 1636cm^{-1} (amide band I) and 1535 cm^{-1} (amide band II).

3.5.2 Polycondensation of *iso*-phthaloyl chloride and ornithine using miscible solvents.

50mL of 0.2M *iso*-phthaloyl chloride were reacted with 50mL of 0.2M ornithine. HCl/0.5M aqueous potassium carbonate solution for 45 minutes according to the general method (sample code ME 3.10). The acetone was removed by rotary evaporation to leave a straw coloured solution. Acidification with concentrated hydrochloric acid gave a white precipitate (yield 84%).

The FT-IR spectrum (Appendix B. Table B3. Spectrum 4) showed strong absorptions at 1701 cm^{-1} (carboxylic acid C=O stretch), at 1636cm^{-1} (amide band I) and at 1535 cm^{-1} (amide band II).

3.5.3 Polycondensation of *iso*-phthaloyl chloride with hexamethylene diamine using miscible solvents.

50mL of 0.2M *iso*-phthaloyl chloride/acetone solution was added rapidly to 50mL of 0.2M hexamethylene diamine/0.7M aqueous potassium carbonate solution. A white precipitate was produced almost instantaneously. The precipitate coagulated into a relatively hard ball and had to be removed to prevent breakage of the beaker (sample code ME 3.11). Stirring was continued for 1 hr with an overhead stirrer at a speed of 2000r.p.m. and more white precipitate was isolated by filtration (combine yield 91%).

The FT-IR spectrum (Appendix B. Table B3. Spectrum 4) of the initial precipitate and the precipitate isolated by filtration at the end of the reaction were identical. Strong absorptions were present at 1637cm^{-1} (amide band I) at 1538cm^{-1} (amide band II) and at 1282cm^{-1} (amide band III)

3.6 COPOLYMERISATION OF *ISO*-PHTHALOYL CHLORIDE WITH LYSINE AND ADDITIONAL DIAMINES

3.6.1 Copolycondensation of *iso*-phthaloyl chloride with lysine and lysine ethyl ester. 2HCl (ratio 1:1) using miscible solvents.

50mL of 0.2M *iso*-phthaloyl chloride/acetone solution was added rapidly to 50mL of 0.1M lysine/0.1M lysine ethyl ester. 2HCl/0.7M potassium carbonate solution with stirring. After stirring for 60 minutes, the precipitated white solid (sample code ME 3.12.1) was removed by filtration and the filtrate was removed (yield 81%). Acidification of the filtrate did not produce more precipitate, though addition of excess water caused the clear solution to become opaque.

The FT-IR spectrum (Appendix B. Table B4. Spectrum 5) of the product showed a strong absorption at 1737cm^{-1} (ester C=O stretch), with a shoulder at 1707 cm^{-1} (carboxylic acid C=O stretch). Strong absorptions were also present at 1636cm^{-1} (amide band I) and 1541cm^{-1} (amide band II).

3.6.2 Copolycondensation of *iso*-phthaloyl chloride with lysine and lysine ethyl ester. 2HCl (ratio 3:1) using miscible solvents.

The previous experiment was repeated, increasing the ratio of lysine to lysine ethyl ester from 1:1 to 3:1 whilst maintaining the overall stoichiometric balance of diamine to diacylchloride (sample code ME 3.12.2). No precipitate was formed after stirring for 60 minutes. Acidification of the mixed solvent gave a white precipitate which was isolated as before (yield 84%).

The FT-IR spectrum (Appendix B. Table B4. Spectrum 5) of the product showed a strong absorption at 1722cm^{-1} (ester and carboxylic acid C=O stretch). Strong absorptions were also present at 1637cm^{-1} (amide band I) and 1533cm^{-1} (amide band II).

3.6.3 Copolycondensation of *iso*-phthaloyl chloride with lysine. HCl and hexamethylene diamine using miscible solvents.

50mL of 0.2M *iso*-phthaloyl chloride/acetone solution was added rapidly to 50mL of 0.1M lysine. HCl/0.1M hexamethylene diamine/0.7M aqueous potassium carbonate (sample code ME 3.13). No precipitate was produced during the course of the reaction, but a white gummy precipitate was isolated on acidification and dried as before (yield 72% based on a 1:1 copolymer).

The FT-IR spectrum (Appendix B. Table B4. Spectrum 5) showed a strong absorption at 1709cm^{-1} (carboxylic acid C=O stretch), at 1637cm^{-1} (amide band I) and 1531cm^{-1} (amide band II)

3.7 POLYCONDENSATIONS BASED ON DIETHYLMALONYL CHLORIDE

3.7.1 Polycondensation of diethylmalonyl chloride lysine ethyl ester using miscible acetone/aqueous potassium carbonate systems.

Diethylmalonyl chloride was used as commercially available model compound for other malonyl chloride derivatives requiring synthesis.

Following the general method 50mL of 0.2M diethylmalonyl dichloride in dry acetone and added rapidly to 50mL of a stirred 0.2M lysine ethyl ester. 2HCl/0.6M potassium carbonate aqueous solution. After the initial effervescence, an off white gummy precipitate formed. Stirring with an overhead stirrer was continued for 1hr after which the precipitate removed and washed with distilled water (sample code ME 3.14). The precipitate was washed with distilled water and dried overnight in a vacuum oven at 55°C. (yield 69%). Addition of water to the acetone/water solution caused further precipitation of a white precipitate.

The FT-IR spectrum (Appendix B. Table B5. Spectrum 6) of both precipitates showed strong absorptions at 1735cm^{-1} (ester C=O stretch), at 1662cm^{-1} (amide band I) and 1529cm^{-1} (amide band II). An additional shoulder was present at 1616 cm^{-1} (primary amine N-H def.)

3.7.2 Polycondensation of diethylmalonyl chloride and lysine free base using a miscible acetone/aqueous sodium hydroxide system.

50mL of 0.2M diethylmalonyl chloride acetone solution were reacted with 50mL of 0.2M lysine free base/0.4 M aqueous sodium hydroxide solution for 1 hr whilst stirring at 2000 r.p.m. with an over head stirrer. The acetone was removed on a rotary evaporator and the remaining opaque aqueous phase acidified with concentrated hydrochloric acid. The solution became slightly more opaque, but a precipitate was not produced (sample code ME 3.15).

FT-IR analysis of the aqueous phase showed absorptions at 1709cm^{-1} (carboxylic acid C=O stretch), at 1640cm^{-1} (amide band I) and at 1530 cm^{-1} (amide band II), indicating that amide formation had occurred. The lack of precipitate would, therefore, suggest that only oligomeric material had been formed. This would be consistent with the use of the more reactive aliphatic acid chloride with a stronger base as acid acceptor.

3.7.3 Polycondensation of diethylmalonyl chloride and lysine free base using a miscible acetone/aqueous potassium carbonate system.

The previous experiment was repeated using with 50mL of 0.2M lysine free base/0.7 M aqueous potassium carbonate (sample code ME 3.16.1). Removal of the acetone left a straw coloured solution which produced a white gummy precipitate on acidification (yield 78%).

The FT-IR spectrum (Appendix B. Table B5. Spectrum 6) showed absorptions at 1724cm^{-1} (carboxylic acid C=O stretch), at 1654cm^{-1} (amide band I) and at 1526 cm^{-1} (amide band II), with an additional shoulder at 1620cm^{-1} (N-H_3^+ def., primary amine salts.).

The experiment was repeated in a baffled 1 L resin flask using 300mL of each phase (sample code ME 3.16.2). Similar results were obtained (yield 74%)

3.8 POLYCONDENSATION BASED ON 1,3-BENZENE DI-SULPHONYL CHLORIDE.

3.8.1 Polycondensation of 1,3-benzene di-sulphonyl chloride and lysine ethyl ester. 2HCl using miscible acetone/aqueous potassium carbonate systems.

Following the general method 50mL of 0.2M 1,3-Benzene di-sulphonyl dichloride acetone solution were reacted with 50mL of 0.2M lysine ethyl ester. 2HCl/0.7M potassium carbonate.

After 1 hr of stirring at 2000 r.p.m. with an overhead stirrer, the acetone water solution was poured into an excess of hexane. A pale blue gummy precipitate was obtained (yield 75%). The product was freely soluble in tetrahydrofuran and acetone (sample code ME 3.17.1).

The FT-IR spectrum (Appendix B. Table B6. Spectrum 7) of the precipitate showed strong absorptions at 1735cm^{-1} (ester C=O stretch), at 1331cm^{-1} (S=O antisymmetric stretch) and 1146cm-^{1} (S=O symmetric stretch). An absorption of medium intensity was observed at 1623 cm^{-1} (primary amine N-H def.)

3.8.2 Polycondensation of with 1,3-benzene di-sulphonyl chloride and lysine free base using miscible acetone/aqueous potassium carbonate systems.

Following the general method 50mL of 0.2M 1,3-Benzene di-sulphonyl dichloride acetone solution was reacted with 50mL of 0.2M lysine free base/0.7M potassium carbonate. After stirring at 2000 r.p.m. with an overhead stirrer for 1hr, the acetone/water solution was acidified and poured into an excess of hexane to produce a white gummy precipitate (yield 27%). The product dissolved in tetrahydrofuran with gentle warming (sample code ME 3.17.2).

The FT-IR spectrum of the precipitate (Appendix B. Table B7. Spectrum 7) showed a strong absorption at 1722cm^{-1} (carboxylic acid C=O stretch) with a broad shoulder at approximately 1620cm^{-1} (N-H_3^+ def., primary amine salts.). This was supported by the low molecular weight of the product (Sec. 5.6). Strong absorptions were present at 1329cm^{-1} (S=O antisymmetric stretch) and at 1146cm^{-1} (S=O symmetric stretch).

4. CHAPTER 4

INTERFACIAL SYNTHESIS

4.1 GENERAL METHODS FOR INTERFACIAL POLYMERISATION

The interfacial technique was used as a convenient and rapid method for the production of high molecular weight polyamides from reactive diacyl chlorides and diamines in the presence of an inorganic acid acceptor.

4.1.1 Interfacial polymerisation with a homogeniser (general method 1)

Small scale preparation of polyamides was carried out in a centrifuge tube (diameter 4cm) with three evenly spaced 0.5 cm indented integral baffles using an Ystral T-1500 ultra torax homogeniser. The homogeniser consisted of a shaft bearing either a two pronged (fine) or four pronged (ultra fine) homogenising head, which rotated within a fine or ultra fine dispersion generator. Alternatively a three blade impeller could be fitted. Homogenisers provide rapid efficient mixing with a relatively high ratio of turbulence to mass flow which results in rapid emulsification of one phase within the other. Interfacial reactions between reactive species are diffusion controlled. Rapid generation of a fine emulsion resulting in a large interfacial area improves the reaction kinetics by reducing the distance required for diffusion. Turbulent flow increases transport by forced convection, whereas mass flow maintains the overall homogeneity of the system by distributing the turbulent eddy currents throughout the bulk.

5mL of the aqueous phase, typically consisting of 0.2M solution of diamine with 0.55-1.6M acid acceptor was added as rapidly as possible to the stirred organic phase, using a syringe. The organic phase typically consisted of 0.25M diacyl chloride in a specified solvent. Stirring speeds were between 2000 and 20000 r.p.m. (power settings 1-10). Unless otherwise stated, the fine dispersion generator and head were used on minimum power setting and the temperature was maintained at 0°C with an ice/water bath.

Polymers bearing predominantly esterified carboxyl side groups precipitated during the course of the reaction. If the side groups were expected to be predominantly free carboxyl groups then the aqueous phase was acidified with concentrated hydrochloric acid to effect precipitation. Any precipitated polymer was isolated by Buchner filtration, washed with distilled water and organic solvent to remove inorganic salts and unreacted diacyl chloride and dried in a vacuum oven overnight at 55°C.

4.1.2 Interfacial polymerisation with an overhead stirrer (general method 2).

For larger scale polymer preparations an overhead stirrer equipped with a marine type propeller at full speed (2000r.p.m.) was used for mixing. The reaction vessel used depended on the scale of the reaction. A 250mL baffled beaker was used for reactions of less than 150mL total volume and a 1 or 2 litre baffled resin flask was used for reactions with volumes up to $600cm^{-3}$ and greater than one litre respectively. Concentrations of reagents were similar to those used in the first method and the stirring speed was fixed at 2000r.p.m. The diameter of the impeller was 2cm with the 250mL beaker and 3cm with the resin flasks. Polymers were isolated as in Sec. 4.1.1.

4.1.3 Interfacial polymerisation with a blender (general method 3).

Waring blenders are commonly employed in interfacial syntheses on a laboratory scale. However, blending was found to cause considerable aeration resulting in the evaporation of volatile organic solvents. This lead to alteration of the phase volume and consequently the concentration ratios. Leaking from the base of the blender and overheating resulting in increased solvent evaporation, were also encountered.

4.2 GENERAL METHODS FOR SYNTHESIS OF DIACID CHLORIDES.

Diacid chlorides that were not commercially available were synthesised according to the general methods described.

4.2.1 Synthesis of diacid chlorides by refluxing with excess thionyl chloride.

0.02 moles of the diacid were refluxed for 4 hours with 0.08 moles of thionyl chloride in a 100mL round bottomed flask equipped with a calcium carbonate guard tube. Excess thionyl chloride was removed by distillation and the diacyl chlorides purified by vacuum fractional distillation on a 10cm Vigreux column. The exception to this was the synthesis of phenylglutaryl chloride, which was a solid at room temperature and was used without further purification. Ultrapure samples of diacid chlorides that are solid at room temperature can be obtained by recrystallisation from anhydrous hexane.

4.2.2 Synthesis of diacid chlorides by refluxing with thionyl chloride in benzene with pyridine.

0.1 moles of diacid were suspended in anhydrous benzene in a 250mL round bottomed flask equipped with a condenser and dekalin bubbler. 15.82g of pyridine were added, causing dissolution of the diacid. 23.79g of thionyl chloride were added dropwise to the solution with stirring. After three hours the solution was filtered in an Atmos™ bag under nitrogen to remove pyridinium hydrochloride crystals and the benzene was removed by distillation. Rigorous exclusion of moisture was required to prevent liquification of the pyridinium hydrochloride and the formation of a two phase system.

4.2.3 Synthesis of diacid chlorides by refluxing with phosphorous pentachloride.

0.05 moles of diacid were dissolved in 60mL of redistilled chloroform in a 250mL round bottom flask equipped with a condenser and dekalin bubbler. The solution was cooled to below 0°C in a salt/ice bath. 20.82g of phosphorous pentachloride was added slowly using a powder funnel whilst maintaining the temperature below 5°C. The mixture was then heated to a gentle reflux until evolution of hydrogen chloride stopped. The chloroform and phosphorous oxychloride were removed by distillation and the crude diacid chloride purified by fractional vacuum distillation on a 10cm Vigreux column.

In all cases the diacyl chlorides contained residual phosphorous oxychloride which reacted violently with water when an interfacial experiment was attempted. Only acid chlorides made by the other two methods were used successfully in interfacial polycondensations.

4.3 ACID CHLORIDE SYNTHESIS

4.3.1 Syntheses of ethylmalonyl chloride.

Ethylmalonyl chloride was synthesised using the excess thionyl method. A brown oil was produced after distillation. Charring of the product occurred towards the end of the distillation.

The FT-IR spectrum of the crude product (Appendix B. Spectrum 8) produced from the reaction of ethylmalonic acid with excess thionyl chloride showed a characteristic absorption at 1793cm^{-1} (C=O stretch, acid chloride), with a shoulder at 1706cm^{-1} (carboxylic acid C=O stretch). A further strong peak at 1227cm^{-1} (carboxylic acid C-O stretch and O-H deflection) confirmed the incomplete conversion of the diacid to diacid chloride. There was an unusual peak present at 2135cm^{-1}. The peak was sharp and in the region normally associated with Si-H, B-H or acetylinic C-H stretching. The most likely cause of this spurious peak was contamination of the product with silicone oil from the oil bath used to heat the refluxing system. The peak was not present in the FT-IR spectrum of the distilled product, but the peaks due to the carboxylic acid were still present.

This would suggest that either that fractionating column was not effective enough to achieve separation of the diacid chloride from the monoacid acid chloride or that the highly reactive diacid chlorides were under going hydrolysis prior to or during analysis.

4.3.2 Syntheses of phenylmalonyl chloride.

Phenylmalonyl chloride was synthesised using the excess thionyl chloride and the phosphorous pentachloride methods. Both methods produced yellow oils after distillation. Charring occurred as with the ethylmalonyl chloride.

The FT-IR spectra of the oils (Appendix B. Spectrum 9) showed a strong absorption at 1792cm^{-1} (acid chloride C=O stretch) and 1694cm^{-1} (carboxylic acid C=O stretch). A strong peak was again present at 2135cm^{-1}.

4.3.3 Syntheses of phenylsuccinyl chloride.

Phenylsuccinyl chloride was synthesised by the phosphorous pentachloride method and the benzene/pyridine/thionyl chloride method. In both cases a dark brown oil was produced.

The FT-IR spectrum of the oil produced by the phosphorous pentachloride method (Appendix B. Spectrum 10) showed strong absorptions at 1772cm^{-1} (anhydride C=O asymmetric stretch) with a weaker absorption at 1863cm^{-1} (anhydride C=O symmetric stretch). A strong absorption is also present at 1290 cm^{-1} (Phosphorous oxy chloride P=O vib.). The FT-IR spectrum of the oil produced by the benzene/pyridine/thionyl chloride method (Appendix B. Spectrum 10) showed strong absorptions at 1772cm^{-1} (anhydride C=O asymmetric stretch) with a weaker absorption at 1863cm^{-1} (anhydride C=O symmetric stretch).

4.3.4 Syntheses of phenylglutaryl chloride.

Phenylglutaryl chloride was synthesised using the benzene/pyridine/thionyl chloride method described in section 4.2.2. On removal of benzene, a viscous brown oil remained which solidified on cooling.

The FT-IR spectrum of the oil (Appendix B. Spectrum 10) showed strong absorptions at 1772cm^{-1} (anhydride C=O asymmetric stretch) with a weaker absorption at 1863cm^{-1} (anhydride C=O symmetric stretch) showed a single strong absorptions at 1787cm^{-1} (acid chloride C=O stretch). No shoulder was present in the region typical of the C=O stretch of the carboxylic acid.

4.4 INTERFACIAL POLYCONDENSATIONS BASED ON 1,3-BENZENE DI-SULPHONYL DICHLORIDE.

Beaumais *et. al.*[60] report the interfacial synthesis of a polyamide based on lysine ethyl ester and 1,3-benzene di-sulphonyl dichloride. The resulting polyamide was subjected to base catalysed hydrolytic cleavage of the ester side groups to leave a weak polyanion. This polymer is reported to undergo a hydrophobically driven conformational transition or hypercoiling phenomena. The authors report that the functional polysulphonamide cannot be synthesised directly from lysine and 1,3-benzene di-sulphonyl dichloride, but give no reason.

The original experiments of Beaumais *et. al.*[60] were repeated using higher reagent concentrations and chloroform as the organic solvent in place of dichloromethane. The direct interfacial synthesis of the polysulphonamide based on lysine was attempted using these conditions and the molecular weight distributions investigated. The results are reported in Sec. 5.6. Summaries of the experiments are listed in Appendix A Table A7.

4.4.1 Interfacial polymerisation of lysine ethyl ester. 2HCl with 1,3-benzene di-sulphonyl dichloride.

50mL of 0.2M 1,3-benzene di-sulphonyl dichloride in chloroform was reacted interfacially with 50mL of 0.2M lysine ethyl ester. 2HCl/0.7M potassium carbonate aqueous solution according to general method 2. Stirring was continued for 1 hour during which a pale blue gummy precipitate adhered to the stirrer blades (sample code ME 4.1.1.1).

The precipitate was removed, washed with distilled water and dried in a vacuum oven overnight at 55°C (yield 63%).

Addition of hexane to the chloroform phase resulted in the precipitation of a solvent-swollen, pale blue mass (sample code ME 4.1.1.2), which was washed and dried as the initial precipitate (yield 29%). A sample of the initial precipitate was placed in a cellulose thimble and extracted with hot chloroform using Soxhlet apparatus (sample code ME 4.1.1.3).

The FT-IR spectra of the two precipitates and the extracted precipitate (Appendix B. Table B6, spectrum 11 showed strong absorptions at 1735cm^{-1} (ester C=O stretch) and at 1324cm^{-1} (sulphonamide C=O stretch) and 1146cm^{-1} (sulphonamide C=O stretch). An additional peak was present in the spectrum of the precipitate obtained from the organic phase on hexane addition at 1623 cm^{-1} (N-H def., primary amine).

4.4.2 Interfacial polymerisation of lysine free base with 1,3-benzene di-sulphonyl chloride.

50mL 0.2M 1,3-benzene di-sulphonyl dichloride in chloroform was reacted interfacially with 50mL of 0.2M lysine/0.7m potassium carbonate aqueous solution according to general method 2. After stirring for 1hour the two phases were separated and the aqueous phase acidified with concentrated hydrochloric acid. This resulted in the precipitation of a white gummy mass (sample code ME 4.1.2) which was washed and dried as in the previous experiment (yield 23%). No precipitate was seen on addition of hexane to the chloroform phase after the initial precipitation. The product was virtually insoluble in acetone and tetrahydrofuran.

The FT-IR spectrum (Appendix B. Spectrum 11) of the precipitate showed strong absorptions at 1726cm^{-1} (carboxylic acid C=O stretch), at 1331cm^{-1} (sulphonamide C=O stretch) and at 1146cm^{-1} (sulphonamide C=O stretch). A peak of medium intensity was present at 1620cm^{-1} ($N\text{-}H_3^+$ def., primary amine salts).

4.5 INTERFACIAL POLYCONDENSATIONS BASED ON *ISO*-PHTHALOYL CHLORIDE.

Iso-phthaloyl chloride has been used as a monomer in interfacial polycondensations to produce polyamides[63-69], polyesters[35], polycarbonates[4] and polyiminocarbonates [48,49]. As an aromatic diacyl chloride, *iso*-phthaloyl chloride is less reactive than aliphatic diacyl chlorides, but shows considerably higher resistance to hydrolysis, whilst being relatively soluble in the aqueous phase. These factors make *iso*-phthaloyl chloride particularly attractive for polycondensations involving diamines bearing additional functional groups which, when charged under the conditions of the reaction, are likely to reduce the availability of the diamine in the organic phase. These conditions are somewhat analogous to polyesterifications of bis-phenols where the reactive bis-phenoxide ion is virtually insoluble in the organic phase[1]. In such reactions accelerators such as phase transfer catalysts are usually added to increase the transport of the bis-phenoxide into the organic solvent. Although successful polymerisations have been carried out when aromatic diacyl chlorides have been used in the absence of accelerators[35] the yields and molecular weights of the final product polymers are always higher when accelerators have been used. *Iso*-phthaloyl chloride was chosen as a suitable diacyl chloride because in a polymer it would be asymmetrically positioned relative to the backbone. Such hydrophobic asymmetry is necessary for a polymer to display amphiphilic properties. Phthaloyl chloride would provide more structural asymmetry than *iso*-phthlaoyl chloride, but in reactions with primary amines, end capping thorough imide formation by reaction of the second acyl group with the secondary amide greatly reduces the molecular weight of the polymer[30].

4.5.1 Interfacial polycondensation of *iso*-phthaloyl chloride with lysine ethyl ester. 2HCl.

The interfacial polycondensation of *iso*-phthaloyl chloride with lysine ethyl ester. 2HCl dihydrochloride was investigated under various experimental conditions. Summaries of the experiments are listed in Appendix A Table A8.

4.5.1.1 Interfacial polycondensation of *iso*-phthaloyl chloride and lysine ethyl ester. 2HCl with sodium hydroxide as the acid acceptor.

50mL of 0.2M *iso*-phthaloyl chloride in CCl_4 was reacted interfacially with 50mL of 0.2M lysine ethyl ester. 2HCl/1.6M aqueous sodium hydroxide solution according to general method 2 for 30 minutes.

No precipitate was produced during the course of the reaction, but on acidification of the aqueous phase, a large amount of white precipitate (sample code ME 4.2.1) was formed (yield 66% based on esterified derivative or 73% based on carboxylic acid derivative).

The FT-IR spectrum (Appendix B. Table B8. Spectrum 12) of the precipitate showed strong absorptions at 1709cm^{-1} (carboxylic acid C=O stretch), 1636cm^{-1} (amide band I) and 1535cm^{-1} (amide band II).

It can be seen that ester hydrolysis occurred either during or after the reaction.

4.5.1.2 Interfacial polycondensation of *iso*-phthaloyl chloride and lysine ethyl ester. 2HCl with sodium carbonate as the acid acceptor.

50mL of 0.2M *iso*-phthaloyl chloride in CCl_4 was reacted interfacially with 50mL of 0.2M lysine ethyl ester. 2HCl/1.6M aqueous sodium carbonate solution according to general method 2 for 30 minutes.

A white gelatinous mass formed almost instantly (sample code ME 4.2.2.1). Mixing was virtually suspended and although the stirrer was moved about in the reaction vessel in an attempt to re-fluidise the mass, no improvement was seen. Filtration of the precipitate, which had occluded both the aqueous and organic solvents, proceeded extremely slowly overnight and CCl_4 could be detected by smell after several days drying in a vacuum oven (yield 87%). Acidification of the aqueous phase produced no further precipitation.

The FT-IR spectrum of the initial precipitate (Appendix B. Table B8) showed strong absorptions at 1737cm^{-1} (ester C=O stretch), 1644cm^{-1} (amide band I) and 1533cm^{-1} (amide band II).

4.5.1.3 Hydrolysis of poly (lysine ethyl ester *iso*-phthalamide) with sodium hydroxide solution.

A sample of poly (lysine ethyl ester *iso*-phthalamide) was heated in 0.2M sodium hydroxide solution for 4 hrs giving a colourless solution. The solution was filtered to remove any undissolved material and acidified to pH 3 to give a white precipitate (sample code ME 4.2.2.2).

The FT-IR spectrum of the precipitate showed strong absorptions at 1707cm^{-1} (COOH, C=O stretch), 1644cm^{-1} (amide band I) and at 1536cm^{-1} (amide band II).

4.5.1.4 Interfacial polycondensation of *iso*-phthaloyl chloride and lysine ethyl ester. 2HCl with potassium carbonate as the acid acceptor.

50mL of 0.2M *iso*-phthaloyl chloride in CCl_4 was reacted interfacially with 50mL of 0.2M lysine ethyl ester. 2HCl/0.7M aqueous potassium carbonate solution according to general method 2.

As in the previous case a white gelatinous precipitate was produced. A small portion of the polymer/solvent precipitate was washed in hexane and the remainder of the precipitated mass added to an excess of acetone and refluxed for 30 minutes to remove low molecular weight oligomers. Coagulation of the polymer occurred rapidly to give a solvent-swollen gummy precipitate (sample code ME 4.2.3.1) which was removed and dried in a vacuum oven at 55°C (yield 72%). The acetone was removed on a rotary evaporator to give a white precipitate(sample code ME 4.2.3.2) which was dried as with the insoluble material (yield 17%).

The FT-IR spectra of the two precipitates (Appendix B. Table B8. Spectrum 12) showed strong absorptions at 1737cm^{-1} (ester C=O stretch), 1644cm^{-1} (amide band I) and 1540cm^{-1} (amide band II). The two white solids were structurally similar and it was assumed that the acetone had extracted low molecular weight oligomers from the precipitated polymer.

The experiment was repeated on a larger scale using 300mL of 0.3M solutions of diacyl chloride and diamine with 1.2M potassium carbonate. The coagulated polymer (sample code ME 4.2.4.1) was recovered from excess acetone without refluxing.

Similar results were obtained as with the smaller scale reaction (yield 84%). A sample of the polymer was taken and Soxhlet extracted with chloroform in an attempt to remove low molecular weight material. After refluxing for 4 hours the polymer (sample code ME 4.2.4.2) was dried according to the general method (yield 46%). On addition of hexane to the organic phase a white gummy precipitate (sample code ME 4.2.4.3) was produced (yield 53%).

4.5.1.5 Interfacial polycondensation of *iso*-phthaloyl chloride and lysine ethyl ester. 2HCl with potassium carbonate as the acid acceptor using chloroform as the organic solvent.

50mL of 0.2M *iso*-phthaloyl chloride in $CHCl_3$ was reacted interfacially with 50mL of 0.2M lysine ethyl ester. 2HCl/0.7M aqueous potassium carbonate solution according to general method 2.

Precipitation did not occur rapidly as when CCl_4 was used as the organic solvent. A gradual thickening of the system occurs eventually giving a white precipitate (sample code ME 4.2.5.1) on filtration (yield 55%). On addition of hexane to the chloroform phase a white gummy precipitate (sample code ME 4.2.5.2) was produced (yield 37%).

The FT-IR spectrum of the two precipitates (Appendix B. Table B9. Spectrum 13) showed strong absorptions at 1737cm^{-1} (ester C=O stretch), 1644cm^{-1} (amide band I) and 1535cm^{-1} (amide band II).

Filtration of the solvent-swollen gelatinous precipitate was extremely time consuming and further samples produced by this method were added to excess acetone. The acetone caused the gelatinous precipitate to coagulate into a gummy ball (sample code ME 4.2.6) which was easily recovered from the reaction vessel (yield 69%). Acetone is a non solvent for the high polymer and caused precipitation from the chloroform in the same way as hexane addition. The reduced yield of product obtained by this method compared to the combined yield of material obtained by the addition of hexane may be due to retention of oligomeric material in the resulting acetone/chloroform solution.

It will be seen in Sec. 5.5 that the chloroform soluble material was of similar molecular weight to the precipitated material and, therefore, precipitation and coagulation in acetone did not lower the overall molecular weight of the product, but gave an overall improved yield compared to a reaction in which no non solvent for the high polymer was added.

4.5.2 Copolymerisation of *iso*-phthaloyl chloride with diamino acids and additional diamines.

Copolymers of *iso*-phthaloyl chloride with lysine or ornithine and an additional diamine were synthesised by the interfacial technique. Summaries of the experiments are listed in Appendix A Table A9.

4.5.2.1 Copolymerisation of *iso*-phthaloyl chloride with lysine ethyl ester. 2HCl and lysine

300mL of 0.2M *iso*-phthaloyl chloride in CCl_4 was reacted interfacially with 300mL 0.1M lysine. HCl/0.1M lysine ethyl ester. 2HCl/1.6M aqueous potassium carbonate solution according to general method 2 for 2 hours.

A white gelatinous precipitate formed (sample code ME 4.3.1) almost immediately and stirring became ineffective. The precipitate was then filtered and washed with distilled water and CCl_4 (yield 9.489g). On acidification of the filtrate a white precipitate (sample code ME 4.3.2) was produced (yield 3.48g).

Approximately half the aqueous soluble precipitate dissolved on washing with acetone leaving a white gummy material whilst all of the initial precipitate was soluble in acetone. On addition of water to the acetone the initially colourless solution produced a cloudy dispersion. The theoretical yield of each homopolymer was calculated to be 8.28g for the lysine homopolymer and 9.12g for the lysine ethyl ester homopolymer.

The FT-IR spectrum of the pre-acidified precipitate (Appendix B. Table B10. Spectrum 14) showed strong absorptions at 1736cm^{-1} (ester C=O stretch), at 1641cm^{-1} (amide band I) and at 1541cm^{-1} (amide band II). The spectrum of the precipitate produced on acidification of the aqueous phase precipitate (Appendix B. Table B10. Spectrum 14) showed absorptions at 1695cm^{-1} (carboxylic acid C=O stretch), at 1641cm^{-1} (amide band I) and at 1541cm^{-1} (amide band II).

4.5.2.2 Copolycondensation of *iso*-phthaloyl chloride with lysine. HCl and hexamethylene diamine

300mL of 0.2M *iso*-phthaloyl chloride in CCl_4 was reacted interfacially with 300mL of 0.1M lysine. HCl/0.1M hexamethylene diamine/1.6M aqueous sodium hydroxide solution according to general method 2 for 2 hours.

A white precipitate (sample code ME 4.4.1) was produced during the reaction (yield 8.2g) and a further precipitate (sample code ME 4.4.2) was obtained on acidification of the aqueous phase (yield 3.04g). The theoretical yield of each homopolymer was calculated to be 8.28g for the lysine homopolymer and 7.38g for the hexamethylene homopolymer.

The FT-IR spectrum of the pre-acidified precipitate (Appendix B. Table B11. Spectrum 15) showed strong absorptions at 1637cm^{-1} (amide band I) and at 1540cm^{-1} (amide band II) with a shoulder at 1701cm^{-1} (carboxylic acid C=O stretch).

It is possible that the lack of a strong peak in the 1700cm^{-1} region from a carboxylic acid peak could be due to the predominance of the carboxylate form, however, no increase in the intensity of the peak at 1701cm^{-1} was seen on washing the precipitate in acidified distilled water.

The spectrum of the precipitate produced on acidification precipitate (Appendix B. Table B11. Spectrum 15) showed absorptions at 1707cm^{-1} (carboxylic acid C=O stretch), at 1636cm^{-1} (amide band I) and at 1541cm^{-1} (amide band II).

4.5.2.3 Copolycondensation of *iso*-phthaloyl chloride with ornithine. HCl and hexamethylene diamine.

50mL of 0.2M *iso*-phthaloyl chloride solution in chloroform were added to 25mL of 0.2M ornithine. HCl/0.7M aqueous potassium carbonate solution whilst stirring at 2000 r.p.m. with an overhead stirrer. 25mL of 0.2M hexamethylene diamine/0.7M aqueous potassium carbonate solution were added immediately after the addition of the organic phase.

On addition of the second phase, the reaction medium thickened and a white precipitate (sample code ME 4.5) was produced. Stirring was continued for 2hrs and the precipitate was isolated. No further precipitation occurred on acidification of the aqueous phase. The precipitate was roughly split into two portions and one portion washed in boiling methanol (sample code ME 4.6.1), the other in boiling chloroform and then acetone (sample code ME 4.6.2). The precipitates were isolated and dried in a vacuum oven at 55°C (combined yield 73%). A single precipitate was also obtained when a hexamethylene diamine/carbonate solution was added rapidly to a lysine/carbonate solution reacting interfacially with a solution of *iso*-phthaloyl chloride in $CHCl_3$ (Sample code ME 4.7).

The FT-IR spectrum of the white precipitates produced (Appendix B. Table B12. Spectrum 16 and Table B11 Spectrum 15) showed strong absorptions at 1636cm^{-1} (amide band I) and 1541cm^{-1} (amide band II). There were additional peaks at 1718cm^{-1} and 1789cm^{-1}.

These peaks were assigned to aromatic anhydride C=O stretching frequencies. Mixed anhydride formation could occur at the early stages of the reaction when diacyl chloride is present in a two fold excess by reaction of acyl chloride ended oligomers or diacyl chloride with the carboxylate pendant groups, however, the peaks in the FT-IR spectrum at 1789cm^{-1} and 1718cm^{-1} are indicative of aromatic anhydrides and so a reaction similar to that in Sec 3.3.6.1 where hydrolysis of the acyl end groups leads to formation of anhydride groups within the backbone may be occurring. In the FT-IR spectrum of the sample washed with methanol (Appendix B. Table B12. Spectrum 17), there was no peak at 1789cm^{-1} whilst a strong peak at 1722cm^{-1} was present. It is postulated that on heating in methanol, the anhydride groups reacted to form a mixture of free carboxyl groups and methyl ester derivatives (Fig. 4.1).

The FT-IR spectrum (Appendix B. Table B12. Spectrum 17) of the methanol washings (sample code ME 4.6.3) showed strong absorptions at 1722cm^{-1} (aryl ester C=O stretch), at 1631cm^{-1} (amide band I) and at 1540cm^{-1} (amide band II). An additional band appeared at 1683cm^{-1} (aromatic carboxylic acid C=O stretch). In the spectrum of the sample washed in chloroform and acetone, the peaks at 1787cm^{-1} was still present and the peak at 1721cm^{-1} appeared as a shoulder on a more intense peak at 1698cm^{-1} (carboxylic acid C=O stretch). Anhydride cleavage was not expected to occur under these conditions, hence the peaks at 1787cm^{-1} and 1721cm^{-1} are still present.

4.5.3 Factors effecting the interfacial polycondensation of *iso*-phthaloyl chloride and ornithine. HCl.

The polycondensation reaction between ornithine. HCl and *iso*-phthaloyl chloride under various experimental conditions was studied to gain a better understanding of the interfacial polyamidation reaction between aromatic diacyl chlorides and aliphatic diamines bearing additional functional groups. The polymers produced were analysed by FT-IR spectroscopy and by gel permeation chromatography (GPC). Summaries of the experiments are listed in Appendix A Table A11.

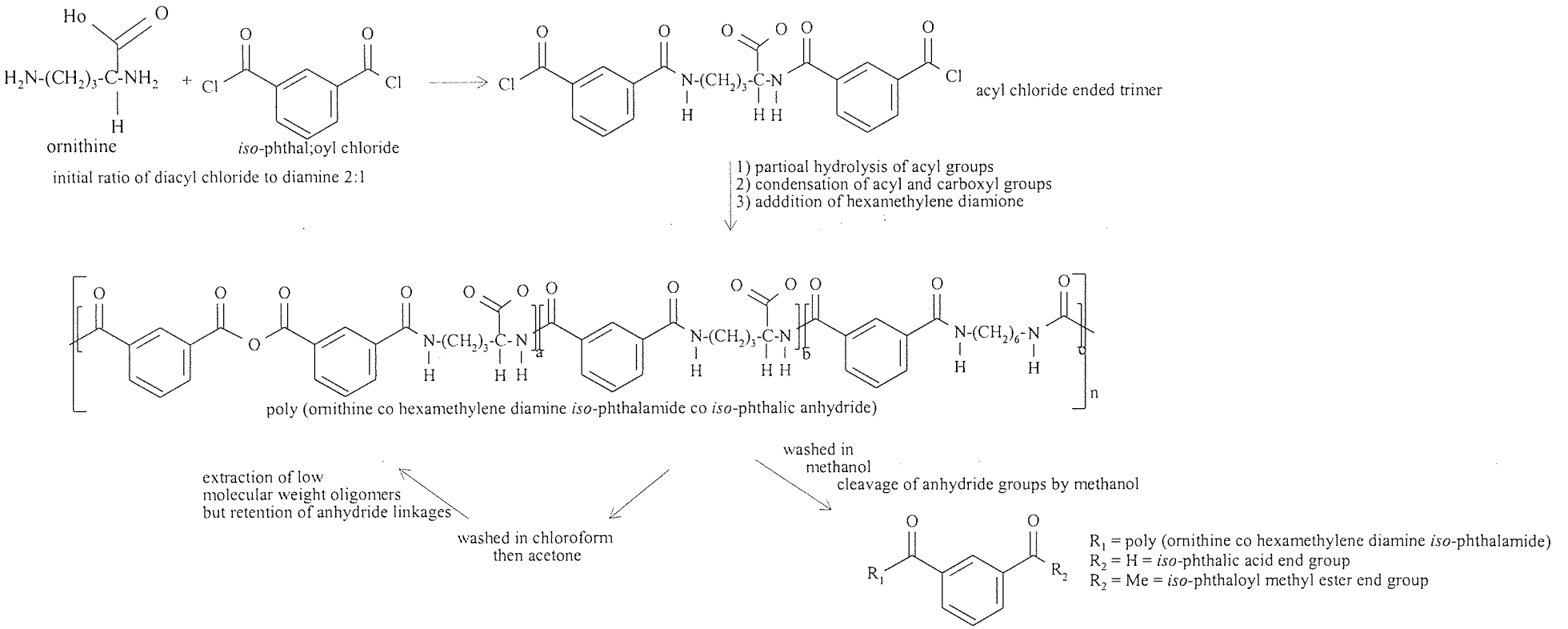


Fig. 4.1. Formation and cleavage of in chain anhydride links in the interfacial synthesis of poly (ornithine co hexamethylene iso-phthalamide).

The molecular weight averages and polydispersisities determined by (GPC) are presented in Ch. 5. Samples sent for molecular weight analysis were washed with distilled water only prior to analysis and were generally found to have broad multi nodal molecular weight distributions. Because of the broad molecular weight distribution and the difficulty encountered in complete removal of solvent, yields for this series of reactions are not reported. Meaningful yields were only obtained from samples washed with either methanol or acetone to remove oligomers and occluded solvents.

4.5.3.1 Investigation into the effect of reaction time on the polycondensation of *iso*-phthaloyl chloride with ornithine. HCl.

5mL of 0.2M ornithine. HCl/1.6M aqueous sodium hydroxide solution was reacted interfacially with 5mL of 0.25M *iso*-phthaloyl chloride solution in CCl_4 according to general method 1 for 5 minutes. The reaction was repeated over 10 and 15 minutes.

In all cases after stirring was suspended, a colourless CCl_4 layer separated out from a white, opaque aqueous phase which produced a white precipitate on acidification (sample codes ME 4.11.1-ME 4.11.3).

4.5.3.2 Investigation into the effect of stirring speed on the polycondensation of *iso*-phthaloyl chloride with ornithine. HCl.

5mL of 0.2M ornithine. HCl/1.6M aqueous sodium hydroxide solution was reacted interfacially with 5mL of 0.25M *iso*-phthaloyl chloride solution in CCl_4 according to general method 1 for 10 minutes with the power setting of the homogeniser at 1. The experiment was repeated with the power setting of the homogeniser at 5 and 10.

In all cases after stirring was suspended, a colourless CCl_4 layer separated out from a white, opaque aqueous phase. A white precipitate was produced on acidification of the aqueous phase (sample codes ME 4.11.4-ME 4.11.6).

4.5.3.3 Investigation into the effect of stirring efficiency on the polycondensation of *iso*-phthaloyl chloride with ornithine. HCl.

5mL of 0.2M ornithine. HCl/1.6M aqueous sodium hydroxide solution was reacted interfacially with 5mL of 0.25M *iso*-phthaloyl chloride solution in CCl_4 according to general method 1 for 10 minutes with the power setting of the homogeniser at 1. The homogeniser was fitted with a fine homogenising head and fine dispersion generator. The experiment was then repeated using a combination of ultrafine homogenising head and fine dispersion generator, with a fine homogenising head and ultrafine dispersion generator and with ultrafine homogenising head and ultrafine dispersion generator.

In all cases after stirring was suspended, a colourless CCl_4 layer separated out from a white, opaque aqueous phase. A white precipitate was produced on acidification of the aqueous phase (sample codes ME 4.11.7-ME 4.11.10).

4.5.3.4 Investigation into the effect of concentration of the aqueous phase on the polycondensation of *iso*-phthaloyl chloride with ornithine. HCl.

5mL of 0.25M *iso*-phthaloyl chloride solution in CCl_4 was reacted interfacially with 4mL of 0.25M ornithine. HCl/2.0M aqueous sodium hydroxide solution according to general method 1. The experiment was then repeated with equivalent amounts of ornithine. HCl and sodium hydroxide with the volume of the aqueous phase reduced to, 3mL, 2mL and 1mL (sample codes ME 4.11.11-ME 4.11.15).

4.5.3.5 Investigation into the effect of concentration of the organic phase on the polycondensation of *iso*-phthaloyl chloride with ornithine. HCl.

5mL of 0.2M ornithine. HCl/1.6M aqueous sodium hydroxide solution was reacted interfacially with 4mL of 0.3125M *iso*-phthaloyl chloride solution in CCl_4 according to general method 1. The experiment was then repeated, reducing the volume of the organic phase to 3mL, 2mL and 1mL (sample codes ME 4.11.16-ME 4.11.20).

4.5.3.6 Investigation into the effect of stoichiometry of the organic and aqueous phase on the polycondensation of *iso*-phthaloyl chloride with ornithine. HCl.

5mL of 0.2M ornithine. HCl/1.6M aqueous sodium hydroxide solution was reacted interfacially with 5mL of 0.25M *iso*-phthaloyl chloride solution in CCl_4 according to general method 1 for 10 minutes with the power setting of the homogeniser at 1. The experiment was then repeated using 5mL of 0.3M, 0.4M, 0.5M and 0.6M ornithine. HCl. The molarity of the sodium hydroxide solution was maintained at 1.6M (sample codes ME 4.11.21-ME 4.11.25).

In an additional experiment, 5mL of 0.2M ornithine. HCl/1.6M aqueous sodium hydroxide solution was reacted interfacially with 5mL of 0.5M *iso*-phthaloyl chloride solution in CCl_4 (sample code ME 4.11.26).

4.5.3.7 Investigation into the effect of organic solvent on the polycondensation of *iso*-phthaloyl chloride with ornithine. HCl.

5mL of 0.2M ornithine. HCl/1.6M aqueous sodium hydroxide solution was reacted interfacially with 5mL of 0.25M *iso*-phthaloyl chloride solution in hexane according to general method 1 for 10 minutes with the power setting of the homogeniser at 1. The experiment was then repeated using toluene and chloroform as the organic solvent (sample codes ME 4.11.27-ME 4.11.29).

4.5.3.8 Investigation into the effect of added sodium chloride on the polycondensation of *iso*-phthaloyl chloride with ornithine. HCl.

5mL of 0.2M ornithine. HCl/1.6M aqueous sodium hydroxide solution, with 1g of sodium chloride, was reacted interfacially with 5mL of 0.25M *iso*-phthaloyl chloride solution in CCl_4 according to general method 1 for 10 minutes with the power setting of the homogeniser at 1. The reaction was repeated using 2 and 3g of added sodium chloride (sample codes ME 4.11.30-ME 4.11.32).

4.5.3.9 Investigation into the effect of concentration of the acid acceptor on the polycondensation of *iso*-phthaloyl chloride with ornithine. HCl.

5mL of 0.25M *iso*-phthaloyl chloride solution in CCl_4 was reacted interfacially with 5mL of 0.2M ornithine. HCl aqueous solution containing sodium hydroxide, according to general method 1 for 10 minutes with the power setting of the homogeniser at 1. The concentration of the sodium hydroxide was decreased from 2.0M to 1.0M in 0.2M steps. 0.2M sodium hydroxide was also used (sample codes ME 4.11.33-ME 4.11.40).

4.5.3.10 Investigation into the effect of nature of the acid acceptor on the polycondensation of *iso*-phthaloyl chloride with ornithine. HCl.

5mL of 0.25M *iso*-phthaloyl chloride solution in CCl_4 was reacted interfacially with 5mL of 0.2M ornithine. HCl/0.8M aqueous sodium carbonate solution according to general method 1 for 10 minutes (sample code ME 4.11.41).

The experiment was repeated using triethylamine as the acid acceptor. Thus, 5mL of 0.25M *iso*-phthaloyl chloride/1.6M triethylamine solution in CCl_4 was reacted interfacially with 5mL of 0.2M ornithine. HCl according to general method 1 for 10 minutes (sample code ME 4.11.42).

The *iso*-phthaloyl chloride solution became yellow and opaque on addition of the triethylamine. The reaction was stopped after 7 minutes because of excessive foaming. When stirring was stopped, a single yellow opaque phase was present.

On acidification, the yellow phase initially turned white and a precipitate settled at the bottom of the reaction vessel, leaving a colourless aqueous phase. No CCl_4 phase was present.

4.5.4 The FT-IR spectrum of poly (ornithine *iso*-phthalamides) synthesised by the interfacial method under various experimental conditions

All the precipitates were analysed by FT-IR spectroscopy. The spectra (examples in Appendix B. Table B13. Spectrum 18 and 19) all showed strong absorptions at 1640 cm^{-1} (amide band I) and at 1542cm^{-1} (amide band II). A strong absorption also appeared in the range 1710cm^{-1} ±10cm^{-1} (carboxylic acid C=O stretch). There are several possible explanations for the variations in the carboxylic acid C=O stretch. Firstly this absorption is particularly sensitive to sample preparation since interaction frequently occurs with alkali metal halides used in the preparation of KBr discs. Thus variations may be expected both between samples examined on a lithium bromide crystal and as KBr discs and between different KBr discs. Inter and intramolecular hydrogen bonding effects the CO vibrational frequency and so molecular conformation will effect the vibrational frequency. Correlations between both νCO and νOH and the pKa of monomolecular acids have been derived by various authors. For unassociated acids Josien suggests the relation νCO=1785.5-10.5pKa. There is an almost constant difference between νCO of unassociated and hydrogen bonded carboxylic acids of 45cm^{-1} and so a similar equation may be applied to hydrogen bonded acids.

Polymeric acids do not posses a fixed pKa, rather a range of apparent pKa values, depending on the degree of ionisation and molecular conformation. It is, therefore, not surprising to find variations in νCO in polymers that are specifically designed to exhibit conformational change.

4.5.5 Investigation into the effect of polyamides based on *iso*-phthaloyl chloride and diamino acids on phase miscibility during interfacial polymerisation.

It was noted during several polymerisations, that the phase volume ratio at the end of the reaction was different to that at the start of the reaction. Indeed in some reactions the organic solvent appeared to have completely disappeared. Initially this was attributed to evaporation of the organic solvent, but this seems unlikely in the case of CCl_4 because of its relatively high boiling point (76-77°C) and the short duration of the experiments.

Morgan[1] noted that polyamides produced by interfacial polymerisation often retained a high portion of the organic solvent, although this observation was restricted to polymers which were formed and precipitated in the organic phase during the course of the reaction. The possibility that the growing polymeric chains could partially solubilise the organic solvent in the aqueous phase was investigated. Summaries of the experiments are listed in Appendix A Table A12.

4.5.5.1 Interfacial polymerisation of *iso*-phthaloyl chloride with ornithine. HCl using sodium hydroxide as the acid acceptor and CCl_4 as the organic solvent.

5mL of 0.2M *iso*-phthaloyl chloride solution in CCl_4 was reacted interfacially for 10 minutes with 5mL of 0.2M ornithine. HCl/1.8M aqueous sodium hydroxide solution according to general method 1 with the exception that no temperature control was used. A white emulsion formed on addition of the aqueous phase, which settled to give a clear CCl_4 phase and an opaque aqueous phase. On acidification of the aqueous phase a white precipitate(sample code ME 4.12.1) was produced (yield 92.2%).

When the experiment was repeated using 1.6M sodium hydroxide solution, a white emulsion is initially formed as before, but foaming also occurred, rising out of the reaction vessel. When stirring was stopped, an opaque white phase remained with what appeared to be a white precipitate at the bottom of the reaction vessel. Approximately 1mL of CCl_4 settled out as clear droplets after 1hour, leaving an opaque white top phase. The opaque phase was removed and acidified to produce a white precipitate (sample code ME 4.12.2) which was dried in a vacuum oven for 4 days at 55°C (apparent yield 126%).

In a separate experiment, 5mL of 2M sodium hydroxide solution was added to the reaction vessel at the end of the reaction and stirred for 30 Seconds. When stirring was stopped, a colourless CCl_4 phase separated out (approximately 3.7mL) from an opaque white phase. On acidification of the opaque phase, a white spongy precipitate (sample code ME 4.12.3) was produced (apparent yield 116%)

The experiment was repeated using 1.4M sodium hydroxide solution. A white emulsion was formed as in the previous two experiments, but no frothing occurred initially. After 2 minutes, frothing began. When stirring was stopped, no phase separation occurred and the foam formed was sufficiently stiff to allow inversion of the reaction vessel. On acidification, a precipitate was formed which coagulated into a ball within the aqueous phase (sample code ME 4.12.4). The precipitate was dried in a vacuum oven at 55°C for a week. A strong characteristic odour from the CCl_4 could still be detected. No yield was taken. In a separate experiment, 5mL of 2M sodium hydroxide solution was added to the reaction vessel at the end of the reaction and stirred for 30 Seconds. When stirring was stopped, a colourless CCl_4 phase separated out (approximately 4.7mL) from an opaque white phase. On acidification of the opaque phase, a white spongy precipitate (sample code ME 4.12.5) was produced (yield 107%).

4.5.5.2 Emulsification of CCl_4 in an aqueous solution of partially neutralised poly (ornithine *iso*-phthalamide).

0.2g of poly (ornithine *iso*-phthalamide) was dissolved in 2mL distilled water with sodium hydroxide. The pH of the resulting solution was 12.1. The aqueous solution was homogenised with 1mL CCl_4 and the pH lowered gradually by the addition of 0.1M hydrochloric acid solution. Stirring was periodically stopped and the emulsion observed to see if phase separation occurred. Eventually, only one opaque phase remained and the pH was measured at 9.7. Phase separation did not occur overnight and acidification of the dispersion caused precipitation of a gummy white precipitate. Since the majority of the organic solvent could be recovered from the aqueous solution of the polymer by increasing the pH after polymerisation it obviously had not evaporated. The initial concentration of the acid acceptor appeared to determine whether or not the CCl_4 was occluded at the end of the reaction.

If the initial pH was too high, then all of the organic solvent remained as a separate phase at the end of the reaction, but as the initial concentration of acid acceptor was reduced more solvent became occluded. As the reaction proceeds, the acid acceptor is consumed by by-product hydrochloric acid and towards the end of the reaction, as the relative excess of diacyl chloride increases, hydrochloric acid will be generated by hydrolysis. As the concentration of acid acceptor falls, eventually the pendant carboxyl groups on the polyamide will become neutralised, allowing the polymer to adopt a less extended conformation. Hydrophobic association may further reduce the radius of gyration. Partially charged hydrophobically associated polymers are known to have amphiphilic properties and thus may be used to solubilise hydrophobic molecules within aqueous systems[126]. Increases in tonicity have been shown to increase the pH at which anionic hypercoiling polymers begin to adopt an amphiphilic structure because of the increasing charge shielding experienced by the carboxylate functions[61]. In addition to the charge shielding effects the presence of a hydrophobic solvent could facilitate a conformational change by increasing the hydrophobic stabilisation within the amphiphile which is the driving force for the conformational change. This could explain why occlusion of the organic solvent into the aqueous phase occurs at pHs significantly higher than the pKa of the carboxylic acid function.

4.5.6 interfacial polymerisation of *iso*-phthaloyl chloride with ornithine. HCl for determination of effect of lithium bromide concentration on apparent molecular weight determined by gel permeation chromatography.

10mL of 0.2M *iso*-phthaloyl chloride solution in CCl_4 was reacted interfacially with 10mL of 0.2M ornithine. HCl/0.8M aqueous sodium hydroxide solution according to general method 1 at speed setting 10. A second sample was prepared using identical conditions with the exception that a three blade impeller was used in place of the fine homogenising head and fine dispersion generator. Both reactions were carried out without temperature control and exhibited large exotherms. The polymer was precipitated by pouring both phases into 50mL of acidified distilled water. The white precipitate was than washed with distilled water and dried (sample codes ME 4.13.1 and ME 4.13.2). Summaries of the experiments are listed in Appendix A Table A13.

4.6 INTERFACIAL POLYCONDENSATIONS BASED ON DIETHYLMALONYL CHLORIDE.

Diethylmalonyl chloride was used as a model diacyl chloride for the synthesised derivatives. Due to the difficulty in preparing and purifying these derivatives a commercially available model compound was used in attempt to identify optimum polymerisation conditions before using the synthesised derivatives. Diethylmalonyl chloride was purchased from the Aldrich Chemical Company and distilled before use. Summaries of the experiments are listed in Appendix A Table A14.

4.6.1 Interfacial polycondensation of diethylmalonyl chloride with lysine ethyl ester. 2HCl.

4.6.1.1 Interfacial polymerisation of diethylmalonyl chloride with lysine ethyl ester. 2HCl using dichloromethane as the organic solvent and sodium hydroxide as the acid acceptor.

10mL 0.2M lysine ethylester/1.6M aqueous sodium hydroxide solution was reacted interfacially with 10mL of 0.2M diethylmalonyl chloride in dichloromethane according to general method 1. Stirring was stopped after 30 minutes and the white emulsion formed settled to give two clear phases (reaction code ME 4.14.1).

The FT-IR spectrum of the aqueous phase showed a strong absorption at 1592cm^{-1} (anti-sym. COO^- vib.) and 1403cm^{-1} (symmetrical COO^- vib.). The absence of the absorptions at 1737cm^{-1} due to the C=O stretch of an ester suggests that the strongly basic aqueous phase has caused hydrolysis of the ester to the carboxylic acid.

4.6.1.2 Interfacial polymerisation of diethylmalonyl chloride with lysine ethyl ester. 2HCl using hexane as the organic solvent and sodium hydroxide as the acid acceptor.

Hexane was used as the organic solvent in order to reduce the partition coefficient of the diacyl chloride in the aqueous phase and, therefore, reduce the rate of hydrolysis.

10mL 0.2M lysine ethylester/1.6M aqueous sodium hydroxide solution was reacted interfacially with 10mL of 0.2M diethylmalonyl chloride in hexane according to general method 1. No precipitate was produced after stirring for two hours (reaction code ME 4.14.2).

The FT-IR spectrum of the aqueous phase showed a strong absorption at 1592cm^{-1} (anti-sym. COO^- vib.) and 1403cm^{-1} (sym. COO^- vib.). There was no absorption at 1737cm^{-1} due to the C=O stretch of an ester.

4.6.1.3 Interfacial polymerisation of diethylmalonyl chloride with lysine ethyl ester using sodium carbonate as the acid acceptor.

10mL 0.2M lysine ethylester/1.6M aqueous sodium carbonate solution was reacted interfacially with 10mL of 0.2M diethylmalonyl chloride in CCl_4 according to general method 1.

The reaction medium gelled instantly producing a white gummy precipitate (sample code ME 4.15.1) with continued stirring (yield 74%). The experiment was repeated using 15mL of each phase (sample code ME 4.15.2) giving a yield of 84%.

The FT-IR spectrum of the precipitate (Appendix B. Table B14) showed strong absorptions at 1735cm^{-1}(ester C=O stretch), at 1654cm^{-1} (amide band I) and at 1526cm^{-1} (amide band II). A peak of medium intensity appears at 1620cm^{-1} (N-H primary amine def.). The presence of absorptions due to primary amines suggests that the product is relatively low molecular weight and is predominantly terminated by lysine residues. The relatively low molecular weight of these products compared to polymers based on aromatic acid chlorides was confirmed by GPC analysis (Sec. 5.6).

The increase in yield was attributed to differences in stirring efficiency since all other conditions were identical. A lowering of the mixing efficiency could delay the initial rapid precipitation sufficiently to allow more complete mixing of the two phases reducing the formation of low molecular weight oligomers in the unstirrable gelatinous mass.

The experiment was repeated according to general method 2 using 50mL of 0.2M lysine ethyl ester. 2HCl/0.7M potassium carbonate and 50mL of 0.2M diethylmalonyl chloride. A white precipitate (sample code ME 4.15.3.1) was produced as before (yield 80%). In this case, although rapid precipitation still occurred, the polymer rapidly coagulated into a ball allowing continued mixing of the two phases. In the smaller scale reaction involving homogenisation of the two phases the polymer was rapidly precipitated in a very dispersed state. Mass flow of the dispersion was effectively halted, limiting efficient mixing to the site around the homogenising head. The mixing efficiency was further reduced by coagulation of the gelatinous precipitate within the dispersion generator. The larger impeller used in the scaled up experiment meant that the emulsion formed on mixing was not as fine so that initial precipitation was not as rapid and the precipitate produced was not as finely dispersed. Additionally the impeller used, a three blade marine type propeller, created a much larger ratio of mass flow to turbulent eddying and thereby increased the overall homogeneity of the system. The slower rates of stirring involved allowed coagulation of the polymer, whereas homogenisers by their very nature cause rapid dispersion of the solid within the liquid. Separation of this finely dispersed polymer from the solvent was difficult and best achieved by centrifuge.

Increasing the volumes of the two phases to 300mL and using an 5% excess of lysine ethyl ester. 2HCl (0.21M compared with the stoichiometric 0.2M) caused a decrease in the yield to 53.5% (sample code ME 4.15.4) with no obvious change in the FT-IR spectrum (Appendix B. Table B14) of the product. The stirring efficiency of this larger system would be decreased relative to the medium scale reaction. Thus, there must be a balance at some point between initial rapid gelation caused by excessive mixing, which results in a disruption of stirring, and inadequate stirring, which results in low molecular weight oligomer formation. Direct comparison between the systems is not possible because in the larger scale polymerisation a 5% excess of lysine ethyl ester. 2HCl was used compared to a stoichiometric balance of reagents in the previous cases and this may exert an effect.

4.6.1.4 Hydrolysis of poly (lysine ethyl ester ethylmalonamide) with sodium hydroxide solution.

A sample of poly (lysine ethyl ester diethylmalonamide) was heated in 1M sodium hydroxide solution for 1 hrs giving a colourless solution. The solution was filtered to remove any undissolved material and acidified to pH 3 to give a white precipitate (sample code ME 4.15.3.2).

The FT-IR spectrum of this solid (Appendix B. Table B15. Spectrum 20) showed strong absorptions in the FT-IR spectrum at 1707cm^{-1} (carboxylic acid C=O stretch), at 1654cm^{-1} (amide band I) and at 1523cm^{-1} (amide band II), with a shoulder at 1618cm^{-1} (N-H_3^+ def., primary amine salts), indicating that hydrolysis of the ester functions to free carboxylic acid functions had occurred.

4.6.2 Interfacial polymerisation of diethylmalonyl chloride with simple diamines.

4.6.2.1 Interfacial polymerisation of diethylmalonyl chloride and hexamethylene diamine using sodium hydroxide as the acid acceptor.

10mL of 0.2M hexamethylene diamine/1.6M aqueous sodium hydroxide solution was reacted interfacially with 10mL of 0.2M diethylmalonyl chloride in CCl_4 according to general method 1. Mixing of the two phases resulted in an almost instantaneous formation of a white gelatinous precipitate (sample code ME 4.16) that was isolated by filtration(yield 82%).

The FT-IR spectrum of the white solid (Appendix B. Table B16. Spectrum 21) showed strong absorptions at 1654cm^{-1} (amide band I), at 1614 cm^{-1} (N-H def., primary amine) and at 1527 cm^{-1} (amide band II).

A strong smell characteristic of hexamethylene diamine was noticed after the precipitate was dried. This fact, together with the absorption in the infra red spectrum due to amine N-H deflection, suggested that the precipitate was substantially terminated by amine groups and was of low molecular weight.

A precipitate was produced despite the fact that 1.6M sodium hydroxide was used as the acid acceptor. Although this undoubtedly resulted in increased hydrolysis of the diethylmalonyl chloride compared to a carbonate solution it suggested that the failure of lysine ethyl ester. 2HCl to produce a precipitated product is not because of the rapid hydrolysis of the diacid chloride. This was supported by the fact that increasing the hydrophobicity of the organic solvent and, therefore, decreasing the phase miscibility of the system and the partition coefficient of the diacyl chloride in the aqueous phase does not improve the reaction. It would appear that hydrolysis of the ester function of lysine ethyl ester. 2HCl occurred in the strongly basic conditions employed. The reaction was then between lysine and diethylmalonyl chloride.

Treatment of 0.02moles of lysine ethyl ester. 2HCl with 50mL of 1.6M sodium hydroxide caused cleavage of the ester functions within ten minutes. This was shown by the shifting of the band due to the carboxyl C=O stretch from 1737 cm^{-1} to 1715cm^{-1} in the FT-IR spectrum.

4.6.3 Interfacial polymerisation of diethylmalonyl chloride with free diamino acids.

4.6.3.1 Interfacial polymerisation of diethylmalonyl chloride and lysine free base using sodium hydroxide as the acid acceptor.

10mL 0.2M lysine/1.6M aqueous sodium hydroxide solution was reacted interfacially with 10mL of 0.2M diethylmalonyl chloride in CCl_4 according to general method 1. After 1hour the aqueous phase was separated and acidified. No precipitate was formed (reaction code ME 4.17.1). No characteristic odour from the acid chloride could be detected.

The FT-IR spectrum of the organic phase showed no discernible absorptions in the functional region. The aqueous phase showed a strong peak at 1715cm^{-1} (carboxylic acid C=O stretch). A broad peak centred at 1631cm^{-1} (overlapping amide band I and N-H_3^+ def., primary amine salts) and a peak at 1533cm^{-1} (amide band II) were also present.

The presence of absorptions due to amide bands I and II indicates that some of the acid chloride reacted with lysine to form oligomeric amides. The lack of acid chloride smell or spectral evidence for its presence coupled with the absence of any precipitate on acidification suggested that hydrolysis of the acyl chloride had occurred. Diethylmalonyl chloride as an aliphatic acid chloride would be relatively reactive compared to aromatic acyl chlorides such as *iso*-phthaloyl chloride. However, its branched nature means that solubility in the aqueous phase would be reduced relative to adipoyl chloride, for example, and so it would be expected to exhibit a higher resistance to hydrolysis than straight chain aliphatic acid chlorides. Reaction with lysine would increase the abstraction of low acyl ended oligomers into the aqueous phase, where rapid hydrolysis would occur, because of the pendant carboxylate groups. Similar results were obtained with hexane as the organic solvent.

4.6.3.2 Interfacial polymerisation of diethylmalonyl chloride and lysine free base with no organic solvent.

0.3941g of diethylmalonyl chloride (0.004m) was added directly to 10mL of 0.2M lysine. HCl/1.6M aqueous sodium hydroxide with rapid stirring. A ratio 2:1 of diacyl chloride to diamine of was used in an attempt to offset hydrolysis. In addition to offsetting hydrolysis, the oligomers formed at the start of the reaction will be predominantly acyl chloride ended and will show minimal solubility in the aqueous phase. No precipitate was produced on acidification (reaction code ME 4.17.2).

The FT-IR spectrum of the acidified aqueous phase showed a broad strong absorptions centred at 1624cm^{-1} (overlapping amide band I and N-H_3^+ def., primary amine salts) with weaker absorptions at 1709cm^{-1} (carboxylic acid C=O stretch) and at 1526cm^{-1} (amide band II).

4.6.4 Investigation of factors effecting the interfacial polymerisation of diethylmalonyl chloride and lysine.

4.6.4.1 Interfacial polymerisation of diethylmalonyl chloride with lysine ethyl ester. 2HCl in the presence of lysine free base.

15mL of 0.2M solution of lysine ethyl ester. 2HCl containing 0.1500g of lysine free base and 2.544g of sodium carbonate (1.6M) was reacted interfacially with 15mL of 0.2M solution of diethylmalonyl chloride in CCl_4 according to general method 1. A white gelatinous precipitate (sample code ME 4.18) was formed almost immediately (yield 46%).

The FT-IR spectrum of the precipitate (Appendix B. Table B16. Spectrum 22) showed strong absorptions at 1741cm^{-1} (ester C=O stretch), 1663cm^{-1} (amide band I), 1620cm^{-1} (N-H def., primary amine) and 1526cm^{-1} (amide band II)

The yield is much lower than when lysine ethyl ester alone is used (Sec. 4.6.1) although the yield is only 9% lower than that obtained when a 20% excess of lysine ethyl ester. 2HCl is used. The lowering of the yield could be due to the stoichiometric imbalance or a salting out effect exerted by the lysine ethyl ester on the lysine making it more accessible for reaction, resulting in the formation of soluble material. The fact that no precipitate was produced on acidification of the aqueous phase would suggest that only oligomeric product was formed by the reaction between lysine and diethylmalonyl chloride.**Interfacial polymerisation of diethylmalonyl chloride with lysine free base followed by lysine ethyl ester.**

15mL of 0.2M lysine. HCl/0.8M sodium carbonate solution was reacted interfacially with 15mL of 0.2M diethylmalonyl chloride in CCL_4 according to general method 1 for 10 minutes. The aqueous phase was then removed and replaced with 15mL of 0.2M lysine ethyl ester. 2HCl/1.0M sodium carbonate solution.

On addition of the second aqueous phase a gel was produced which slowly coagulated to give a white precipitate (sample code ME 4.19) which was removed by filtration (yield 23%). Acidification of the first aqueous phase produced no precipitate.

The FT-IR spectrum of the precipitate (Appendix B. Table B16. Spectrum 22) showed a strong but relatively broad band centred at 1722cm^{-1} (ester and carboxylic acid C=O stretch) with a strong absorption at 1657cm^{-1} (amide band I) and 1534cm^{-1} (amide band II). A shoulder was present on the amide band I absorption at 1620cm^{-1} (N-H def., primary amine) Examination of the initial aqueous phase showed absorptions at 1709cm^{-1}(carboxylic acid C=O stretch), 1644cm^{-1} (amide band I) and 1533cm^{-1} (amide band II). These results indicated oligomer formation in the first reaction since although no precipitate is obtained on acidification of the first aqueous phase the FT-IR spectrum suggested the presence of amide linkages. Also the yield of polymer in the second reaction was reduced compared to direct interfacial reaction of a lysine ethyl ester/carbonate solution with a solution of diethylmalonyl chloride in CCl_4.

4.6.4.3 Interfacial polymerisation of diethylmalonyl chloride and lysine. HCl using chloroform as the organic solvent.

10mL of 0.2M diethylmalonyl chloride in chloroform was reacted interfacially with 10mL of 0.2M lysine. HCl/1.6M aqueous sodium hydroxide solution according to general method 1. After 10 minutes the emulsion formed was allowed to settle and the aqueous phase acidified. A small amount of coagulated white/grey precipitate formed but disappeared on mixing of the two phases leaving a white opaque aqueous phase and a colourless organic phase. No precipitate was isolated by filtration (reaction code ME 4.20.1).

The FT-IR spectrum of both phases showed strong absorptions at 1709cm^{-1} (carboxylic acid C=O stretch), 1659 (amide band I) and 1527cm^{-1} (amide band II).

In experiments involving the polymerisation of lysine ethyl ester. 2HCl with *iso*-phthaloyl chloride it was found that when choroform and dichloromethane were used as the organic solvent a portion of the product remained dissolved in the organic phase and could be precipitated by addition of a non solvent such as hexane or CCl_4. When lysine was used as the diamine all of the product was found in the aqueous phase because the pendant carboxylic acids are all in the carboxylate form throughout the pH range of the reaction.

Acidification of the aqueous phase converts the carboxylate ions to the carboxylic acid form. The hydrophilicity of the polymer (or oligomers) is thus reduced and abstraction into the organic phase may have occurred.

4.6.4.4 Interfacial polymerisation of diethylmalonyl chloride and lysine. HCl with varying amounts of sodium carbonate as the acid acceptor using dichloromethane as the organic solvent.

10mL of a 0.2M solution of diethylmalonyl chloride in dichloromethane was reacted interfacially according to general method 1 with 10mL of an 0.2M aqueous solution of lysine. HCl containing 1.6M, 1.2M or 0.8M sodium carbonate. The two phases were stirred for 10 minutes and then the aqueous phase was acidified. In all cases a white emulsion was formed on mixing of the two phases which settled after stirring was stopped to give two clear phases. Acidification caused the aqueous phases to become cloudy in the first two cases and resulted in a small amount of precipitate in the last case (yield 5%). In all cases films formed at the interface overnight (sample codes ME 4.21.1-ME 4.21.3).

The FT-IR spectrum of the aqueous phases and the precipitates all showed peaks at 1709cm^{-1} (carboxylic acid C=O stretch), 1657cm^{-1} (amide band I) and at 1526cm^{-1} (amide band II). A shoulder was also present at 1624cm^{-1} (NH_3^+ def., primary amine salts).

4.6.4.5 Interfacial polymerisation of diethylmalonyl chloride and lysine. HCl with a phase volume ratio of 2.1.

10mL of 0.2M diethylmalonyl chloride in chloroform was reacted interfacially with 5mL of 0.4M lysine. HCl/1.6M sodium carbonate for 10 minutes. On acidification a small amount of grey/white precipitate (sample code ME 4.20.1) was obtained (yield 9%). On standing overnight a film formed at the interface.

The FT-IR spectrum of the aqueous phases and the precipitates all showed strong absorptions at 1709cm^{-1} (carboxylic acid C=O stretch), 1657cm^{-1} (amide band I) with a shoulder at 1624cm^{-1} (N-H_3^+ def., primary amine salts) and at 1526cm^{-1} (amide band II).

4.6.5 Effect of accelerators on the interfacial polymerisation of diethylmalonyl chloride with diamino acids and their derivatives.

The effect of surfactants and phase transfer agents was investigated. Surfactants reduce the interfacial tension of the two phases allowing a finer emulsion and, therefore, more interfacial area to be generated. Reducing the interfacial tension also increases the rate of transport of material across the interface. Phase transfer catalysts such as alkyl quaternary ammonium salts may be surface active, depending on their structure, but generally act by replacing the gegen ion of an ionised species e.g. the sodium ion in a bis-phenoxide during polyesterifications. The aliphatic or aromatic groups on the quaternary ammonium ion confer a degree of solubility on the ion pair in the organic phase and thus increase the availability of the ion for reaction. The activity of the ion is increased since its solvent cage is removed, leaving a naked ion. This presents a potential problem in the case of lysine since the polymer forming reaction is between the amine functions and the acyl chloride functions. Enhancing the reactivity of the carboxylate ion could lead to competing anhydride formation. Summaries of the experiments are listed in Appendix A Table A15.

4.6.5.1 Interfacial polymerisation of diethylmalonyl chloride and lysine ethyl ester. 2HCl with 1% sodium laurate.

15mL of 0.2M solution of diethylmalonyl chloride in CCl_4 was reacted interfacially with 15mL of 0.2M lysine ethyl ester. 2HCl/1.6M aqueous sodium carbonate solution containing 1% w/w sodium laurate according to general method 1. Rapid gelation of the system occurs but the gel remained stirrable compared to the same reaction without added surfactant. The gel gradually coagulated to give a white precipitate (sample code ME 4.22) which was isolated by filtration (yield 81%).

The FT-IR spectrum of the solid (Appendix B. Table B17. Spectrum 20) produced showed strong absorptions at 1741cm^{-1} (ester C=O stretch), 1663cm^{-1} (amide band I), 1526cm^{-1} (amide band II). Additional peaks appear at 1572cm^{-1} and 1383cm^{-1}. These were assigned to the anti-sym. and symmetrical stretch of the ionised carboxyl group of residual sodium laurate. No peak appeared in the region of the spectrum associated with NH deflection of primary amines.

The yield obtained was slightly lower than if surfactant was not used. Stirring efficiency was improved as was evidenced by the continual fluidity of the stirred system. This would lead to an increase in yield and average molecular weight. Reducing the interfacial tension would not only increase transport of diamine into the organic phase but also diacyl chloride into the aqueous phase where it would undergo hydrolysis. It would seem, therefore, that any benefit from increased stirring efficiency is offset by a simultaneous increase in the hydrolysis of the diacyl chloride.

Homogenisers rapidly create a large amount of interfacial area allowing fast reaction between reagents at the locus of polymerisation. and so increases in stirring efficiency may be of less importance compared to the reduction of interfacial tension. In systems where stirring is less effective, such as in general method 2, or where access of the diamine to the organic phase is restricted then addition of a surfactant may be advantageous.

Kondo *et. al.*[68-71] found that polyamides from adipoyl chloride and lysine were formed in highest yields and with highest average molecular weight using high speed stirring with surfactants added to the aqueous phase. In this case abstraction of adipoyl chloride into the aqueous phase and subsequent hydrolysis is already rapid and the reduction in interfacial tension would have a beneficial effect on the transport of lysine into the organic phase

4.6.5.2 Interfacial polymerisation of diethylmalonyl chloride and lysine free base with 1% sodium laurate.

10mL of 0.2M diethylmalonyl chloride in CCl_4 was reacted interfacially with 10mL of 0.2M lysine free base/1.6M aqueous sodium hydroxide solution containing 1% w/w sodium laurate for 10 minutes according to general method 1 for 10 minutes.

A white emulsion was formed which persisted after stirring was stopped. Considerable foaming occurred during the stirred period. Acidification of the emulsion did not produce any precipitate although any oligomeric polyamide produced may have been solublised by the surfactant (reaction code ME 4.23).

The FT-IR spectrum of the emulsion (Appendix B. Table B17. Spectrum 23) showed a strong absorption at 1715cm^{-1} (carboxylic acid C=O stretch) and 1533cm^{-1} (amide band II). A broad peak centred at 1600cm^{-1} is also present (amide band I with overlapping C=O stretch and COO^- anti-symmetric stretch)

4.6.5.3 Interfacial polymerisation of diethylmalonyl chloride and lysine with 1% benzalkonium bromide.

10mL of 0.2M diethylmalonyl chloride in dichloromethane was reacted interfacially with 10mL of 0.2M lysine ethyl ester. 2HCl/1.6M aqueous sodium carbonate solution containing 1% w/w benzalkonium bromide according to general method 1 for 10 minutes. The reaction foamed whilst stirring and no phase separation occurred when stirring was stopped.

On acidification the two phases separated out to give a colourless organic phase and an opaque aqueous phase. No precipitate was formed and it was assumed the reaction had been unsuccessful (sample code ME 4.24.1). However, by comparison with the results from the polymerisation of diethylmalonyl chloride with lysine using chloroform as the solvent where the initial precipitate dissolved on mixing of the two phases it is possible that low molecular weight polymer was formed but was solublised by the organic phase as it was neutralised. In the case where no phase transfer catalyst was used and chloroform was the solvent initially two distinct phases were present and dissolution of the polymer only occurred on mixing of the two phases. In this case only one phase was present on acidification and mixing would not be necessary to bring the polymer into contact with the organic solvent.

The FT-IR spectrum of the emulsion (Appendix B. Table B17. Spectrum 23) showed strong absorptions at 1637cm^{-1} (amide band I), 1579cm^{-1} (COO^- anti-symmetric stretch), 1533cm^{-1} (amide band II) and 1409cm^{-1} (COO^- anti-symmetric stretch). The fact that the pendant carboxyl functions are still ionised suggest that the pH may not have been lowered sufficiently to cause complete neutralisation.

In phase transfer catalysed polyesterifications the phase transfer catalyst is regenerated in the organic phase when the phenoxide ion is acylated and returns to the aqueous phase where it can complex with more phenoxide ion and transport them across the interface. In the case of lysine, however, the phase transfer agent is complexed with the carboxylate anion which is not involved in the main polymer forming reaction and so the catalyst is not regenerated. The phase transfer agent would need to be present in equimolar quantities with the lysine in order to transfer all of the lysine to the organic phase.

The experiment was repeated using 100mL of 0.2M diethylmalonyl chloride and 100mL of 0.2M lysine/0.2M benzalkonium bromide/0.7M aqueous potassium carbonate solution. After stirring for 2 hours the system was acidified. No precipitate was produced (reaction code ME 4.24.2).

4.6.5.4 Interfacial polymerisation of diethylmalonyl chloride with lysine. HCl in the presence of 18-crown-6.

100mL of diethylmalonyl chloride in dichloromethane was reacted interfacially with 100mL of 0.2M lysine. HCl/1.6M aqueous potassium hydroxide solution containing 0.02 moles of 18-crown-6 according to general method 2.

After stirring for 45 minutes a sample was taken from the reactor. Two cloudy phases were present, becoming clearer on acidification. Stirring was continued for 3 hours. Acidification of a sample withdrawn from the cloudy aqueous phase produced no precipitate (reaction code ME 4.25.1).

When left overnight (without stirring) a colourless precipitate was seen in the aqueous phase (sample code ME 4.25.2). The gel like precipitate was removed and when the aqueous phase was acidified further a white precipitate was produced (sample code ME 4.25.3).

The FT-IR spectrum of the dried "gel" (Appendix B. Table B18. Spectrum 24) showed a relatively weak absorption as a shoulder at 1820cm^{-1} (anhydride C=O symmetric vib.) with an absorption of medium intensity at (1765cm^{-1} anhydride C=O asymmetric vib). Strong absorption were also present at 1717cm^{-1} (C=O carboxylic acid C=O stretch), at 1663cm^{-1} (amide band I) and 1530cm^{-1} (amide band II). The FT-IR spectrum of the white precipitate produced on acidification of the aqueous phase(Appendix B. Table B18. Spectrum 24) showed a strong broad absorption centred at 1719cm^{-1} (Carboxylic acid C=O stretch) with additional strong peaks at 1256 and 1162cm^{-1} (C-O stretch aliphatic carboxylic acid). The FT-IR spectrum of the white precipitate was almost identical to that of ethylmalonic acid suggesting that the precipitate was in fact hydrolysed acid chloride

4.6.5.5 Interfacial polymerisation of diethylmalonyl chloride with lysine. HCl in the presence of polyethylene glycol.

100mL of 0.2M diethylmalonyl chloride in dichloromethane was reacted interfacially with 100mL of 0.2M lysine. HCl/1.6M aqueous potassium hydroxide solution containing 0.02 moles of PEG 1000, 2000 or 3000 according to general method 2 for 2 hours. In all cases no precipitation was produced on acidification (reaction codes ME 4.26.1-ME 4.26.3).

4.7 INTERFACIAL POLYCONDENSATIONS BASED ON DODECANEDIOYL DICHLORIDE.

Dodecanedioyl dichloride is a highly reactive aliphatic acid chloride but its long chain length reduces its solubility in the aqueous phase, compared to the shorter chain aliphatic acid chlorides such as glutaryl chloride, and thus reduces the rate of hydrolysis. The diacyl chloride was investigated as a method of introducing a flexible, hydrophobic aliphatic chain into the backbone of a polyanionic polyamide. In an aqueous system as the degree of neutralisation of the pendant carboxylate ions is increased the electrostatic repulsion along the backbone decreases. The aliphatic sections may collapse into the interior of a mono molecular micellular structure at some intermediate degree of neutralisation before the polymer precipitates out of solution provided that are sufficiently hydrophobic. Summaries of the experiments are listed in Appendix A Table A16.

4.7.1 Interfacial polymerisation of Dodecanedioyl dichloride with lysine.

4.7.1.1 Interfacial polymerisation of dodecanedioyl dichloride and lysine free base with sodium hydroxide as the acid acceptor and CCl_4 as the organic solvent.

5mL of 0.25M dodecanedioyl dichloride in CCl_4 was reacted interfacially with 5mL of 0.2M lysine free base/1.6M aqueous sodium hydroxide solution according to general method 1 for 10 minutes.

A white emulsion formed on stirring and foam was seen to rise up the reaction vessel. A large exotherm was noted with the temperature rising to 47°C. When stirring was stopped three layers were initially present, a colourless organic and aqueous phase with a milky white phase in between, eventually clearing to two phases. A plug of white gummy precipitate (sample code ME 4.27) was produced on acidification of the aqueous phase. The measured yield was in excess of the theoretical yield (apparent yield 127%). After drying the precipitate was washed with methanol and the methanol insoluble fraction dried overnight in a vacuum oven at 55°C (yield 96%).

The FT-IR spectrum of the white precipitate (Appendix B. Table B19) showed strong absorptions at 1696cm^{-1} (carboxylic acid C=O stretch), at 1637cm^{-1} (amide band I) and at 1540cm^{-1} (amide band II). The methanol extract also showed these peaks indicating that the methanol soluble material was structurally similar to the methanol insoluble material, presumably low molecular weight oligomeric material.

4.7.1.2 Interfacial polymerisation of dodecanedioyl dichloride and lysine free base with sodium hydroxide as the acid acceptor and hexane as the organic solvent.

5mL of 0.25M dodecanedioyl dichloride in hexane was reacted interfacially with 5mL 0.2M lysine free base/1.6M aqueous sodium hydroxide solution according to general method 1 for 10 minutes.

Whereas in the previous experiment a white frothy emulsion was formed almost instantaneously, there seemed to be a "lag" period of about two minutes during which the reaction medium gradually thickened whilst remaining clear. The reaction medium suddenly turned white and began to froth as in the previous case. On acidification a white precipitate (sample code ME 4.28) was obtained (apparent yield 130%).

The FT-IR spectrum of the white precipitate (Appendix B. Table B19. Spectrum 25) showed strong absorptions at 1701cm^{-1} (COOH, C=O stretch), at 1637cm^{-1} (amide band I) and at 1540cm^{-1} (amide band II).

4.7.2 Interfacial polymerisation of dodecanedioyl dichloride with ornithine. HCl.

4.7.2.1 Interfacial polymerisation of dodecanedioyl dichloride and ornithine. HCl with sodium hydroxide as the acid acceptor and CCl_4 as the organic solvent.

5mL of 0.25M dodecanedioyl dichloride in CCl_4 was reacted interfacially with 5mL 0.2M ornithine. HCl/1.8M aqueous sodium hydroxide solution according to general method 1 for 10 minutes. A white gummy precipitate (sample code ME 4.29) was produced on acidification (apparent yield 113%).

The FT-IR spectrum of the white precipitate (Appendix B. Table B19 spectrum 25) showed strong absorptions at 1696cm^{-1} (COOH, C=O stretch), at 1644cm^{-1} (amide band I) and at 1540cm^{-1} (amide band II).

4.7.2.2 Interfacial polymerisation of dodecanedioyl dichloride and ornithine. HCl with sodium hydroxide as the acid acceptor and hexane as the organic solvent.

5mL of 0.25M dodecanedioyl dichloride in CCl_4 was reacted interfacially with 5mL 0.2M ornithine. HCl/1.8M aqueous sodium hydroxide solution according to general method 1 for 10 minutes.

At the end of the reaction two phases were present, a slightly opaque aqueous bottom phase and a foamy opaque top phase. There was very little odour of hexane. On acidification, two clear, colourless phases appeared and a coagulated white precipitate (sample code ME 30) was produced (apparent yield 135%).

The FT-IR spectrum of the white precipitate (Appendix B. Table B19. Spectrum 25) showed strong absorptions at 1701cm^{-1} (COOH, C=O stretch), at 1637cm^{-1} (amide band I) and at 1540cm^{-1} (amide band II).

The apparently high yields could be accounted for by the presence of a large fraction of low molecular weight material or dodecanedioic acid or residual solvent. An alternative possibility is the formation of branched imides from the reaction of the excess diacyl chloride with the secondary amides in the backbone of the polymer which would increase the molecular weight of the repeat unit. Since the yield after extraction with methanol is still close to 100 the large excess cannot be entirely due to low molecular weight oligomers since removal of these would give a much reduced overall yield. Washing in methanol would not be expected to remove high molecular weight branched material and no evidence for imide formation is found in the FT-IR spectra of these precipitates. The excess must, therefore, be either residual solvent or hydrolysed diacylchloride which would be insoluble in the acidic aqueous phase. Hydrolysis would be expected to be lower when hexane is used as the organic solvent as the diacid chloride would have a higher partition for the organic solvent. From the polymerisations of dodecanedioyl dichloride with ornithine it can be seen that the relative excess is higher when hexane is the organic solvent rather than CCl_4.

Morgan[1] noted that interfacially synthesised polyamides retained large amounts of organic solvent in cases where the polymers were precipitated from the organic phase during the course of the reaction. In the polymerisations described above the polymer remains soluble in the aqueous phase and it would appear that the polymer has an ability to partially solubilise the organic solvent within the aqueous phase. Organic solvent is still detectable even when subjected to drying under vacuum at elevated temperature indicating that the solvent is strongly associated with the polymer.

4.8 INTERFACIAL POLYCONDENSATIONS BASED ON PHENYLMALONYL CHLORIDE.

Phenylmalony chloride was investigated as a monomer capable of introducing a more hydrophobically asymmetric moiety onto the polyamide backbone. Summaries of the experiments are listed in Appendix A Table A17.

4.8.1 Interfacial polymerisation of phenylmalonyl chloride and lysine ethyl ester.

4.8.1.1 Interfacial polymerisation of phenylmalonyl chloride and lysine ethyl ester. 2HCl with sodium carbonate as the acid acceptor.

10mL of 0.2M phenylmalonyl chloride in CCl_4 was reacted interfacially with 10mL of 0.2M lysine ethyl ester. 2HCl/1.6M aqueous sodium carbonate solution according to general method 1 for 10 minutes.

On stirring rapid precipitation of a yellow solid (sample code ME 4.31) was seen (yield 40%). If stirring was continued for 30 minutes the initially yellow precipitate became red (sample code ME 4.32.1) and adhered to the blades of the stirrer (yield 45%). The precipitate was very sticky and isolation of all the product was difficult. The yields refer to the amount of precipitate that was isolated and not necessarily the amount produced during the reaction although effort was made to minimise any difference.

The FT-IR spectrum of the precipitates (Appendix B. Table B20. Spectrum 26) showed strong absorptions at 1737cm^{-1} (ester C=O stretch), 1668cm^{-1} (amide band I) and 1525cm^{-1} (amide band II). A weak peak was present at 1596cm^{-1} (COO^- anti-symmetric stretch).

4.8.1.2 Hydrolysis of poly (lysine ethyl ester phenylmalonamide) with sodium hydroxide solution.

A sample of poly (lysine ethyl ester phenylmalonamide) was heated in 0.2M sodium hydroxide solution for 4 hrs giving a pale yellow solution. The solution was filtered to remove any undissolved material and acidified to pH 3 to give a yellow precipitate (sample code ME 4.32.2).

The FT-IR spectrum of the precipitate (Appendix B. Table B20. Spectrum 26) showed strong absorptions at 1711 cm^{-1} (COOH C=O stretch) and at 1668 cm^{-1} (amide band I).

4.8.1.3 Interfacial polymerisation of phenylmalonyl chloride and lysine ethyl ester. 2HCl with sodium carbonate as the acid acceptor in the presence of 1% sodium laurate.

15mL of 0.2M phenylmalonyl chloride solution in CCl_4 was reacted interfacially with 15mL of 0.2M lysine ethyl ester. 2HCl/1.6M aqueous sodium carbonate solution containing 1% w/w sodium laurate according to general method 1 for 10 minutes.

A yellow emulsion was formed that remained completely stirrable for the duration of the experiment. A yellow gummy precipitate (sample code ME 4.33) was isolated by filtration (yield 47%).

The FT-IR spectrum of the precipitate (Appendix B. Table B21. Spectrum 26) showed strong absorptions at 1737cm^{-1} (ester C=O stretch), 1668cm^{-1} (amide band I) and 1525cm^{-1} (amide band II). A weak peak was present at 1596cm^{-1} (COO^- anti-symmetric stretch).

4.8.2 Interfacial polymerisation of phenylmalonyl chloride with simple diamines.

4.8.2.1 Interfacial polymerisation of phenylmalonyl chloride and hexamethylene diamine with sodium carbonate as the acid acceptor.

10mL of 0.1M phenylmalonyl chloride solution in CCl_4 was reacted interfacially with 10mL of 0.2M hexamethylene diamine/1.6M aqueous sodium hydroxide solution according to general method 1 for 10 minutes.

An excess of diamine was used to increase the partition of the diamine into the organic phase in order to reduce hydrolysis. It was felt that reducing the concentration of the phenylmalonyl chloride, rather than increasing the concentration of the diamine, would result in more efficient stirring because of the reduced amount of precipitate.

The initial yellow emulsion rapidly turned pink. A fine pink precipitate (sample code ME 4.34) was isolated by centrifugation. The pink precipitate became yellow on drying (yield 65% based on phenylmalonyl chloride)

The FT-IR spectrum of the precipitate (Appendix B. Table B21. Spectrum 27) showed strong absorptions at 1663cm^{-1} (amide band I) and 1540cm^{-1} (amide band II) with a weak absorption at 1598cm^{-1} (COO^- anti-symmetric stretch). Examination of the aqueous phase after removal of the precipitate showed similar absorptions whilst examination of the organic phase showed an additional absorption at 1715cm^{-1} (carboxylic acid C=O stretch). This confirms the assignment of the absorption at 1598cm^{-1} since the carboxylic acid would be expected to be uncharged in the non polar solvent. The carboxyl function must originate from the phenylmalonyl chloride since hexamethylene diamine contains no such functions. The presence of this peak in the polymers based on lysine ethyl ester can, therefore, also be attributed to hydrolysis of the diacyl chloride rather than the protective ester function on the diamino acid.

4.8.3 Interfacial polymerisation of phenylmalonyl chloride with free diamino acids.

4.8.3.1 Interfacial polymerisation of phenylmalonyl chloride and ornithine with sodium carbonate as the acid acceptor.

10mL of 0.2M phenylmalonyl chloride in CCl_4 was reacted interfacially with 10mL of 0.2M ornithine/1.6M aqueous sodium hydroxide solution according to general method 1 for 10 minutes. A very small amount of yellow/brown precipitate is produced on acidification of the aqueous phase.

The precipitate was soluble in dichloromethane and chloroform indicating that it was low molecular weight. Insufficient sample was available for The FT-IR spectrum.

4.9 INTERFACIAL POLYCONDENSATIONS BASED ON PHENYLGLUTARYL CHLORIDE.

Phenylglutaryl chloride was investigated as a method of increasing the backbone flexibility whilst maintaining the increased hydrophobic asymmetry afforded by phenylmalonyl chloride. Summaries of the experiments are listed in Appendix A Table A18.

4.9.1 Interfacial polymerisation of phenylglutaryl chloride and lysine ethyl ester. 2HCl.

4.9.1.1 Interfacial polymerisation of phenylglutaryl chloride and lysine ethyl ester. 2HCl with sodium carbonate as the acid acceptor.

300mL of 0.1M phenylglutaryl chloride solution in chloroform was reacted interfacially with 300mL of 0.1M lysine ethyl ester. 2HCl/0.4M aqueous sodium carbonate solution according to general method 2 for 30 minutes.

A fine pink/white precipitate (sample code ME 4.36.1) was formed on stirring and was isolated by centrifugation. On drying the precipitate became brown (yield 48%).

The FT-IR spectrum of the precipitate (Appendix B. Table B22. Spectrum 28) showed strong absorptions at 1735cm^{-1} (COOH, C=O stretch), 1637cm^{-1} (Amide band I) and at 1546cm^{-1} (amide band II). An additional peak was present at 1616cm^{-1} (N-H def., primary amine end groups).

4.9.1.2 Hydrolysis of poly (lysine ethyl ester phenylglutamide) with sodium hydroxide solution.

A sample of poly (lysine ethyl ester phenylglutamide) was heated in 0.2M sodium hydroxide solution for 4 hrs giving a brown solution. The solution was filtered to remove any undissolved material and acidified to pH 3 to give a brown precipitate (sample code ME 4.36.2).

The FT-IR spectrum of the precipitate (Appendix B. Table B22. Spectrum 28 showed strong absorptions at 1737cm^{-1} (COOH, C=O stretch), 1637cm^{-1} (amide band I) and at 1546cm^{-1} (amide band II).

4.9.2 Interfacial polymerisation of phenylglutaryl chloride with free diamino acids.

4.9.2.1 Interfacial polymerisation of phenylglutaryl chloride and lysine. HCl with sodium carbonate as the acid acceptor.

30mL of 0.1M phenylglutaryl chloride in chloroform was reacted interfacially with 30mL of 0.1M lysine. HCl./0.4M aqueous sodium carbonate solution according to general method 2 for 10 minutes.

When stirring was stopped the orange emulsion formed, settled to give an orange organic phase and a colourless aqueous phase with a orange sludge at the interface. On acidification an orange precipitate (sample code ME 4.37) was produced (yield 32%).

The FT-IR spectrum of the orange solid (Appendix B. Table B23. Spectrum 28) showed strong absorptions at 1715 cm^{-1} (COOH, C=O stretch), 1637cm^{-1} (amide band I) and at 1546cm^{-1} (amide band II). An additional peak was present at 1611cm^{-1} ($N\text{-}H_3^+$ def., primary amine salts).

4.9.2.2 Interfacial polymerisation of phenylglutaryl chloride and lysine. HCl with sodium hydroxide as the acid acceptor.

20mL of 0.2M phenylglutaryl chloride (method 2) in CCl_4 was reacted interfacially with 30mL of 0.1M lysine. HCl./1.6M aqueous sodium hydroxide solution according to general method 2 for 15 minutes.

A brown solid (sample code 4.38) was obtained on acidification of the orange emulsion (yield 19.8%).

The FT-IR spectrum of the orange solid showed strong absorptions at 1715 cm^{-1} (COOH, C=O stretch), 1637cm^{-1} (amide band I) and at 1546cm^{-1} (amide band II). An additional peak was present at 1616cm^{-1} ($N\text{-}H_3^+$ def., primary amine salts).

4.9.2.3 Interfacial polymerisation of phenylglutaryl chloride and lysine. HCl with benzene as the organic solvent and sodium hydroxide as the acid acceptor

20mL of 0.2M phenylglutaryl chloride (method 3) in chloroform was reacted interfacially with 20mL of 0.2 M lysine. HCl./1.6M aqueous sodium hydroxide solution according to general method 2 for 10 minutes.

A brown precipitate (sample code ME 4.39) was obtained as in the previous experiment although the precipitate appeared more crumbly than when chloroform and CCl_4 were used as the organic solvents (yield 14.6%). It would appear that benzene, although a better solvent for phenylglutaryl chloride, is a poorer solvent for the polymer produced by the reaction of phenylglutaryl chloride with lysine. This would explain the drop in yield as the diacyl chloride would have a lower partition towards the aqueous phase and the oligomers formed would precipitate at lower molecular weight than if CCl_4 was the solvent.

The FT-IR spectrum of the brown solid showed strong absorptions at 1715 cm^{-1} (COOH, C=O stretch), at 1637cm^{-1} (amide band I) and at 1546cm^{-1} (amide band II) and an additional peak at 1618cm^{-1} ($N\text{-}H_3^+$ def., primary amine salts).

4.9.2.4 Interfacial polymerisation of phenylglutaryl chloride and ornithine. HCl with sodium carbonate as the acid acceptor.

20mL of 0.1M phenylglutaryl chloride (method 3) in chloroform was reacted interfacially with 20mL of 0.1M ornithine. HCl./0.5M aqueous sodium carbonate solution according to general method 2 for 10 minutes.

An orange emulsion forms on stirring which does not settle on standing. On acidification of the emulsion an orange organic phase and a colourless aqueous phase separate out and a small amount of orange/pink precipitate (sample code ME 4.40) was obtained (yield 7%).

The FT-IR spectrum of the pink/orange solid (Appendix B. Table B22. Spectrum 29) showed strong absorptions at 1714cm^{-1} (COOH, C=O stretch), at 1636cm^{-1} (amide band I) and at 1544cm^{-1} (amide band II).

4.10 INTERFACIAL POLYCONDENSATIONS BASED ON ITACONYL CHLORIDE.

Interfacial polymerisations based on itaconyl chloride were investigated as a route to potentially cross linkable biodegradable functional polymers. Hydrogels that respond to pH have been investigated as drug delivery devices but are generally not biodegradable. Such functional polymers offer an alternative method for the synthesis of biodegradable microparticles. An emulsion of the dissolved polymer could be crosslinked to produce insoluble microspheres. Alternatively the polymer could be applied as an aqueous solution containing a photo initiator and crosslinked to an insoluble gel. The water content of the gel could be varied by controlling the hydrophilic/hydrophobic balance of the polymer. Summaries of the experiments are listed in Appendix A Table A10.

4.10.1 Interfacial polymerisation of itaconyl chloride and lysine ethyl ester. 2HCl

4.10.2 Interfacial polymerisation of itaconyl chloride and lysine ethyl ester. 2HCl with sodium carbonate as the acid acceptor.

15mL of 0.2M itaconyl chloride in CCl_4 was reacted interfacially with 15mL of 0.2M lysine ethyl ester. 2HCl/1M sodium carbonate aqueous solution according to general method 1 for 15 minutes.

From the brown emulsion that forms, a yellow/brown precipitate (sample code ME 4.8) is produced (yield 59%). The experiment was repeated using chloroform and dichloromethane as the organic solvents (sample codes ME 4.9 and ME 4.10). A light yellow precipitate was produced in both cases (yields 38% and 36% respectively).

The FT-IR spectrum of the solid (Appendix B. Table B24. Spectrum 30) showed strong peaks at 1734cm^{-1} (ester C=O stretch), 1648cm^{-1} (amide band I) and at 1556 and 1543cm^{-1} (amide band II). The splitting of the band was attributed to the electron withdrawing effect of the double bond asymmetrically positioned within the itaconic moiety exerting a stronger effect on the α amide compared to the β amide. An additional shoulder at 1702cm^{-1} (carboxylic acid C=O stretch) was present when chloroform and dichloromethane were used as solvents.

On acidification of the aqueous phases additional precipitation occurred. The peak at 1702cm^{-1} is in the C=O stretching region of a carboxylic acid and was assigned to the C=O stretch of carboxylic acid end groups rather than to that of hydrolysed ester functions since both the reaction times and the concentration of the acid acceptor are less than in other polymerisations where the ester linkage was unaffected. The purity of the acid chloride was low, being only 90% technical grade. This would have undoubtedly effected the yields and molecular weight distribution of the polymers produced.

4.11 INTERFACIAL SYNTHESES BASED ON ETHYLMALONYL CHLORIDE.

Ethylmalonyl chloride was used as a monomer to introduce the weakly hydrophobic ethyl group into the backbone of the polyamide whilst retaining the structural asymmetry required for hydrophobic aggregation. Summaries of the experiments are listed in Appendix A Table A19.

4.11.1 Interfacial polymerisation of ethylmalonyl chloride lysine ethyl ester. 2HCl.

4.11.1.1 Interfacial polymerisation of ethylmalonyl chloride lysine ethyl ester using sodium carbonate as the acid acceptor.

10mL 0.2M lysine ethyl ester. 2HCl/1.6M aqueous sodium carbonate solution was reacted interfacially with 10mL of 0.2M ethylmalonyl chloride in CCl_4 according to general method 1.

The reaction medium gelled instantly producing a white gummy precipitate (sample code ME 4.41.1) with continued stirring (yield 69%).

The FT-IR spectrum of the precipitate (Appendix B. Table B25. Spectrum 31) showed strong absorptions at 1737cm^{-1}(ester C=O stretch), at 1650cm^{-1} (amide band I) and 1530cm^{-1} (amide band II). As with polycondensates of diethylmalonyl dichloride and lysine ethyl ester. 2HCl a peak of medium intensity appears at 1620cm^{-1} (NH_2 N-H def.).

4.11.1.2 Hydrolysis of poly (lysine ethyl ester ethylmalonamide) with sodium hydroxide solution.

A sample of poly (lysine ethyl ester ethylmalonamide) was heated in 0.2M sodium hydroxide solution for 4 hrs giving a colourless solution. The solution was filtered to remove any undissolved material and acidified to pH 3 to give a white precipitate (sample code ME 4.41.2).

The FT-IR spectrum of the precipitate showed strong absorptions at 1711 cm^{-1} (COOH C=O stretch) and at 1648 cm^{-1} (amide band I).

5. CHAPTER 5

GEL PERMEATION CHROMATOGRAPHY

5.1 GEL PERMEATION CHROMATOGRAPHY.

Gel permeation chromatography is a chromatographic technique that separates molecules on the basis of hydrodynamic volume. As such, the technique is dependent on the solvational and conformational properties of the analytes and can be very sensitive to the presence of additives as will be seen in Sec. 5.2. G.P.C. was used principally to investigate the molecular weight distributions of samples of poly (ornithine *iso*-phthalamides) and poly (lysine ethyl ester *iso*-phthalamides) synthesised by interfacial polycondensation or by mixed phase solution polycondensation using miscible solvents. The molecular weight distributions of aliphatic functional polyamides are also presented. It was found that the apparent molecular weights of functional polyamides bearing pendant carboxyl groups were extremely sensitive to the presence of impurities and added salts such as lithium bromide and ammonium acetate in the mobile phase. Salts such as these are normally added to the mobile phase when using polar solvents to prevent unwanted non-steric interactions between the sample and the column packing material. The long term reproducibility of the results has proved to be unreliable and so direct comparisons are only made between chromatograms of samples submitted for analysis at the same time. Similar trends can, however, be identified between sets of experiments run at different times. Unless stated otherwise polyamide samples were analysed in dimethylformamide at 80°C using a 30cm polystyrene-co-di-vinyl benzene column with a differential refractometer detector. Molecular weights are expressed as polyethylene oxide/polyethylene glycol(PEO/PEG) equivalents. In view of the chemical difference between the samples and the calibrants there may be considerable differences between the PEO/PEG equivalents and the actual molecular weights of the samples since chemically dissimilar analytes have their own hydrodynamic volume to molecular mass relationships.

The polysulphonamide samples were found to be soluble in THF and were analysed using an in-house system with THF as the solvent on a similar column. Molecular weights are expressed as PEO/PEG equivalents and the previous comments about chemical dissimilarity between calibrants and samples apply.

Gel permeation chromatography is a secondary technique and is most usefully employed in a comparative manner between chemically similar species rather than as an absolute measure of molecular weight. The technique proved to be rather problematic with certain samples, notably the poly (ornithine *iso*-phthalamide) samples, and reproducibility between repeat runs of solutions of the samples and between fresh solutions was poor. Possible factors effecting the reproducibility are discussed in Sec. 5.2. and Sec. 5.4.2.

5.2 EFFECT OF LITHIUM BROMIDE ON APPARENT MOLECULAR WEIGHT DISTRIBUTION OF POLYAMIDES BASED ON ORNITHINE.

Lithium bromide is routinely added to the mobile phase in gel permeation chromatography when a polar solvent is used in order to prevent unwanted non steric effects such as ionic interactions between the solute and the column packing and intermolecular aggregation. Exclusion effects have been observed in the absence of lithium bromide (or a similar salt) or when insufficient salt was present. Such exclusion effects lead to repulsion of the sample from the packing pores causing low elution volumes thus giving apparently higher molecular weights. It was assumed, not proved, that the chromatography of samples was carried out in the absence of such non-steric interactions.

Following initial problems of poor reproducibility between repeat runs of samples using standard amounts of lithium bromide (0.01%) the amount of lithium bromide was increased according to the instructions of the column manufacturers. This led to significant decreases in apparent molecular weight initially suggesting that the polymer molecules were less favourably solvated, i.e. had adopted a less solvent swollen, more compact structure thus reducing their hydrodynamic volume. In the absence of lithium bromide the apparent molecular weight increased dramatically but reproducibility was still low. The low reproducibility was attributed to the affore mentioned non steric effects. Similar results were obtained with *N*-methyl-yrolidone as the solvent.

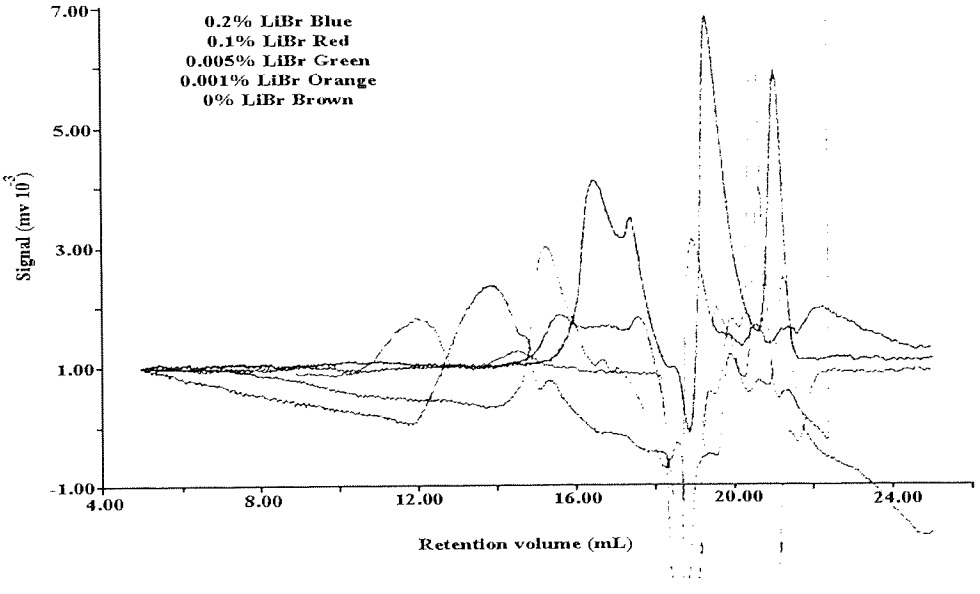
In order to determine the effect of lithium bromide on the apparent molecular weight of poly (*iso*-phthalamides) based on ornithine, two samples of interfacially synthesised poly (ornithine *iso*-phthalamide) were analysed by G.P.C. using dimethylformamide containing 0%, 0.001%, 0.005%, 0.01% and 0.02% lithium bromide. Each sample was run in duplicate and was reanalysed a week later. No calibrations were obtained for the initial runs and so comparison can only be made between the raw chromatograms. In the second week calibrations were made for two lithium bromide concentrations (0.005% and 0.2%). The molecular weight averages are shown along with the polydispersisities in Table 5.1.

Table 5.1 Variation in molecular weight and polydispersity of poly (ornithine *iso*-phthalamide) determined by G.P.C. in DMF with concentration of lithium bromide.

Samples Analysed 20.12.93				
Sample (RAPRA code) (sample code)	% LiBr	Mw 1st run 2nd run 3rd run	Mn 1st run 2nd run 3rd run	Mw/Mn 1st run 2nd run 3rd run
POI3 ME 4.13.1	0.02	2080 1770 1410	1160 1140 990	1.8 1.6 1.4
POI5 ME 4.13.2	0.02	1740 1520 1430	1070 1040 1020	1.6 1.5 1.4
POI3 ME 4.13.1	0.005	1690 2310 2590	800 1160 1240	2.1 2.0 2.1
POI5 ME 4.13.2	0.005	1350 1610 1830	760 900 1020	1.8 1.8 1.8

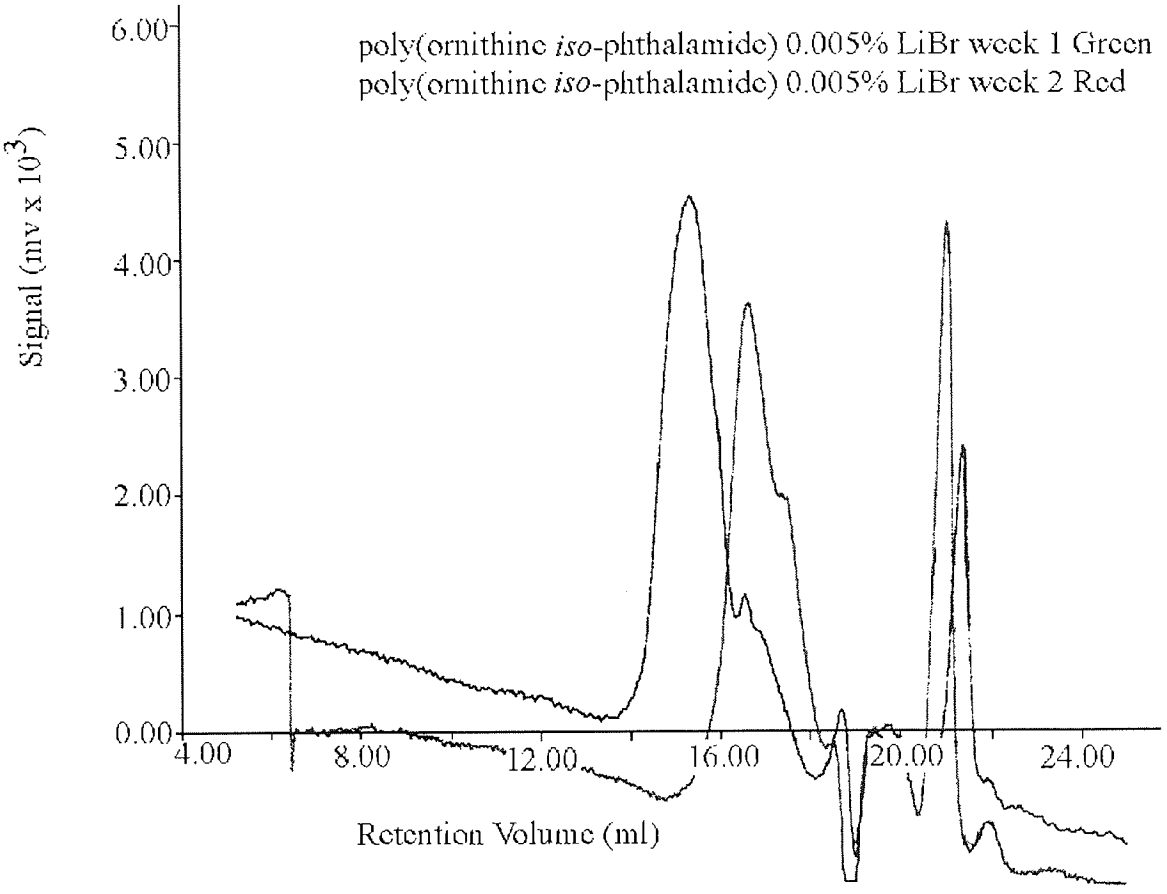
Comparison of the initial chromatograms of the samples run in the first week with varying concentrations of lithium bromide (Fig. 5.1) showed a decrease in retention time (corresponding to increasing molecular weight) with a decrease in the amount of lithium bromide and a consistent shift to earlier retention times (apparently higher molecular weight) for the second run. Conversely, the calibrated chromatograms run in the second week showed a decrease in retention time (corresponding to an increase in the apparent molecular weight) with an increase in the lithium bromide concentration. In addition, the sample with the lower lithium bromide concentration (0.005%) showed an increase in apparent molecular weight on the second and third run as in the initial work, whereas the sample with the higher lithium bromide concentration (0.02%) showed a decrease in apparent molecular weight on the second and third run. The relation between the apparent molecular weights of the two samples on the second and third run followed the trend set in the first week, i.e. higher apparent molecular weight with a lower concentration of lithium bromide.

Fig. 5.1 Raw chromatograms of poly (ornithine iso-phthalamide) with varying concentrations of lithium bromide (sample POI5).



The problem of interpretation of the results was complicated by a set of positive and negative system peaks appearing at higher retention volume (low molecular mass) and by the fact that the observed retention volumes for the sample run in 0.005% and 0.02% lithium bromide in the second week were very different (apparently higher, corresponding to lower molecular mass) to those observed on the initial run (Fig. 5.2).

Fig. 5.2 Raw chromatograms of poly (ornithine iso-phthalamide) with 0.005% lithium bromide from week 1 and week 2.



The complex set of peaks at high retention volume could result from dissolved gases, lithium bromide and moisture as well as low molecular weight polymer. Each chemical species will exhibit a specific relationship between detector response and concentration, therefore, it is not possible to estimate the relative amounts of low molecular weight material from the peak area unless the species are known to be chemically similar. Even if chemical similarity can be assumed there may be some detector bias towards low molecular weight material since at low molecular weight refractive index is no longer independent of molecular weight. It is obvious that the apparent molecular weight is very dependent on the salt concentration used and the apparently complex results were attributed to the fact that the system exhibited a long lived memory effect, with the performance of the column being dependent on the history of samples previously run.

The apparent drift up in molecular weight would suggest a slowly decreasing lithium bromide content, whilst the drift down in apparent molecular weight being would suggest increasing lithium bromide content, based on the observations made with varying lithium bromide concentrations. Lithium bromide at the required concentration was pumped through the system for some time before the samples were run. However, in view of a possible memory effect this time period may have been insufficient. It was suggested that the system could be stabilised by running a particular lithium bromide concentration for a sufficient time period prior to running the sample.

It is possible that the two key features of these chromatograms, i.e. the fall in apparent molecular weight with increasing amount of lithium bromide and the changes in apparent molecular weight on successive runs with constant amounts of lithium bromide are the result of two separate but related phenomena. It is known that structurally similar polymers such as poly (lysine *tera*-phthalamide) bind cations to various extents depending on the nature of the cation[60,71]. It is also well established that relatively hydrophobic polymers containing electron donating groups can act as phase transfer catalysts by chelating cations in a polar hydrophilic phase and transferring the complexed cation and its counterion into an immiscible hydrophobic phase in a mode of action identical to the crown ethers. A conformational change would inevitable accompany the chelation process to allow optimum interaction of the electron donating groups with the cation. The polymer chain would contract around the cation thus reducing its hydrodynamic volume, leading to higher retention volume, corresponding to an apparent decrease in molecular weight. Increasing the concentration of salt would increase the effect and hence lead to an apparent fall in molecular weight with increasing complex formation. This effect is reproducible and so is more likely to be due to a polymer salt interaction than a polymer column interaction which may be effected by the history of the column. Non steric effects could explain the apparent rise in molecular weight on successive runs with identical salt concentrations. The shift to earlier retention time in the second and third runs of the samples could reflect a gradual clogging of the pores on successive runs due to hydrophobic interaction of the chelated polymer ion complex.

Such a complex would essentially be an inverse micelle with a hydrophilic centre and a hydrophobic surface. The phenyl group of polystyrene co di-vinyl benzene beads are used as hydrophobic binding sites in some hydrophobic interaction columns used in protein and peptide purification. The technique relies on the differences in the hydrophobicity between solutes and thus the strength of hydrophobic interaction of the solute and column material. Hydrophobic affinity chromatography is a high performance liquid chromatography technique and although the surface area of column material in such systems is much greater than in gel permeation columns one might still expect hydrophobic interaction of the solute with the column material to occur in the gel permeation system.

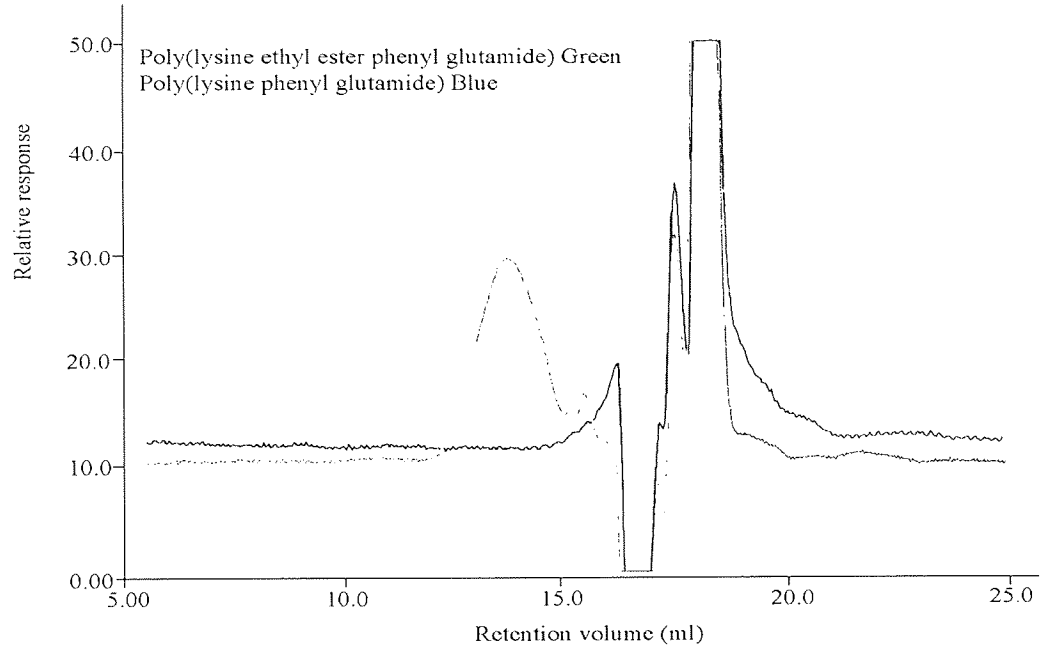
Whilst sample preparation was constant between consecutive runs, differences in sample preparation may have occurred between runs carried out in the first and second week. It would be expected that a greater and hence more time consuming conformational change would occur in the case where a higher lithium bromide concentration was used. Thus the apparent increase in retention time in the sample with the higher lithium bromide content run in the second week could be due to the fact that the polymer solution was run before the conformational change of the polymer, in response to the presence of a cation, was complete. This theory is supported by the fact that the apparent molecular weights of the samples run in the presence of 0.02% lithium bromide in the second week are higher than those run in the presence of 0.005% lithium bromide, whereas it was previously found that the apparent molecular weight decreased in the presence of higher concentrations of lithium bromide.

Since no calibrations were obtained for the initial chromatograms, a comparison between the apparent molecular weights of identical samples run on the two separate occasions could not be made. Such a comparison would show if the apparent molecular weight of the sample run with 0.02% lithium bromide in the second week was higher than that in the first week indicating incomplete conformational transition.

Although the packing material is essentially crosslinked polystyrene, suggesting a hydrophobic material, the materials are often pre-treated in an undisclosed manner by the manufacturers. Ionic interaction between the samples and the column packing cannot be ruled out since such treatment may increase the number of active sites in the material. Lithium bromide suppresses such ionic interactions and decreasing the concentration of the added salt may increase such interaction. A progressive saturation of these active sites at low salt concentrations would explain increases in the apparent molecular weight on successive runs. Ionic interaction of the sample with the column does not explain why the apparent molecular weights of the two samples with high lithium bromide concentrations run on the second week decrease in molecular weight on successive runs. It is likely that several phenomena were occurring simultaneously.

When samples of poly (lysine phenylglutamide) and poly (lysine ethyl ester phenylglutamide) were analysed by GPC there was effectively no detector response for the poly (lysine phenylglutamide) sample. Closer examination of the chromatogram (Fig. 5.3) indicated that there may be a late eluting peak, possibly representing the polymer, which is swamped by a large negative peak at a retention volume of around 17mL.

Fig. 5.3 Raw chromatograms of poly (lysine phenylglutamide) and poly (lysine ethyl ester phenylglutamide).



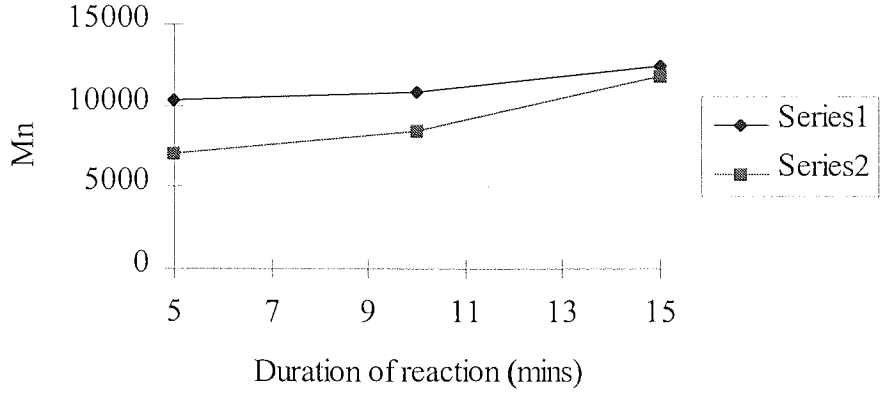
The absence of a detector response indicated that the sample was probably binding strongly with the column packing meaning that no separation based on steric exclusion took place. On the other hand with poly (lysine ethyl ester phenylglutamide) no such interaction occurs and a bimodal molecular weight distribution is obtained. Possible interaction of the polymer with a cation would be reduced by esterification of the carboxyl function and this may explain why polyamides based on esterified diamino acids proved less problematic to analyse by GPC.

5.3 EFFECT OF REACTION CONDITIONS ON THE MOLECULAR WEIGHT AVERAGES AND POLYDISPERSISITIES OF POLY (ORNITHINE *ISO*-PHTHALAMIDE) SYNTHESISED BY INTERFACIAL POLYCONDENSATION.

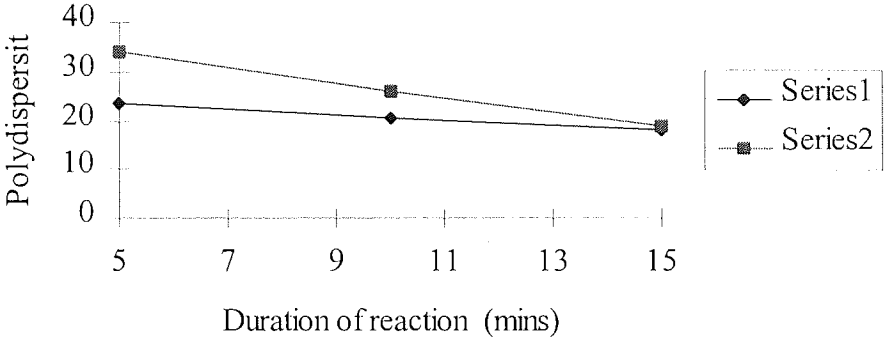
5.3.1 Reaction time (ME 4.11.1-ME 4.11.3).

As the reaction time was increased the number average molecular weight increased, the polydispersity decreased (graphs 5.1 and 5.2). and there was an initial decrease in the weight average molecular weight followed by a slight increase (Graph 5.3).

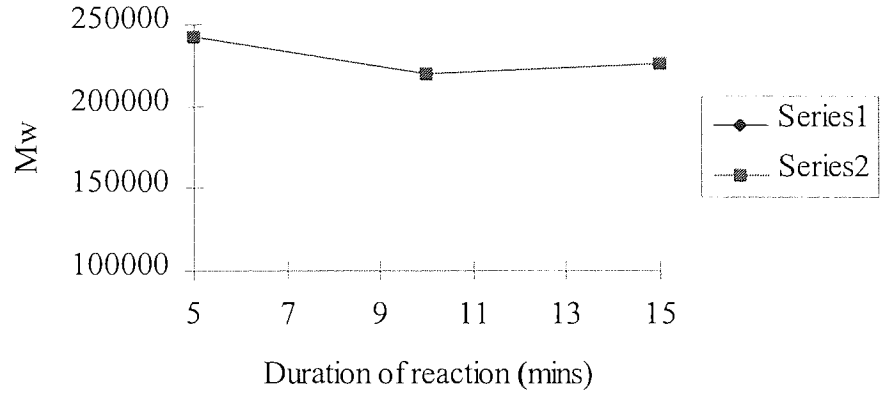
Graph 5.1 Variation in Mn of interfacially synthesized poly (ornithine *iso*- phthalamide) with reaction time.



Graph 5.2 Variation in polydispersity of interfacially synthesized poly (ornithine *iso*- phthalamide) with reaction time.



Graph 5.3 Variation in Mw of interfacially synthesized poly (ornithine *iso*- phthalamide) with reaction time.

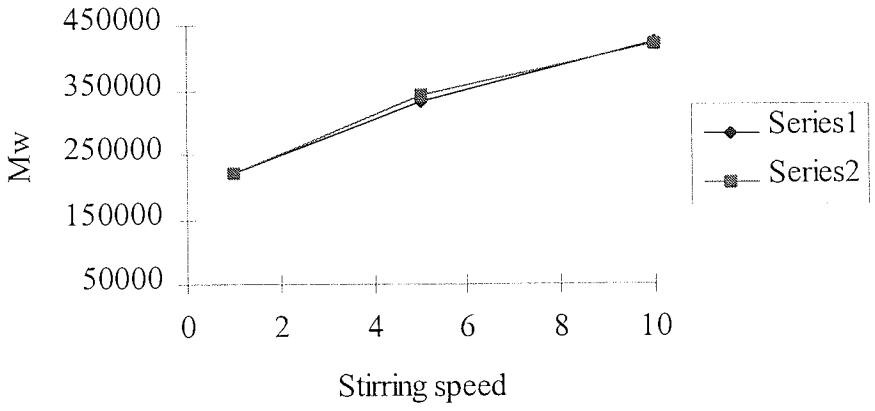


5.3.2 Stirring speed (ME 4.11.4-ME 4.11.6).

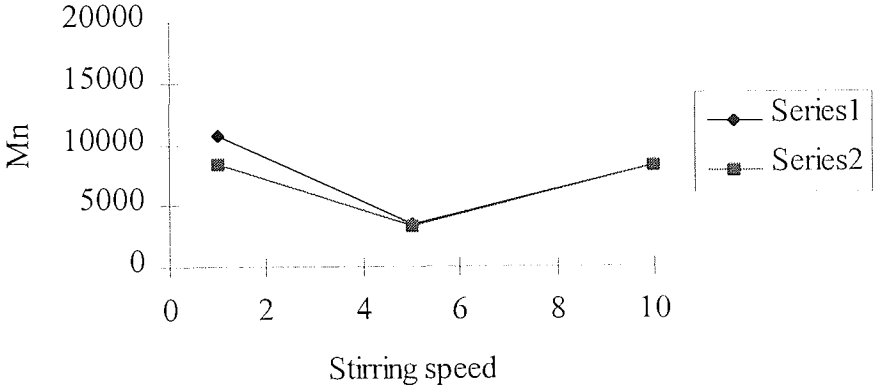
Stirring is vitally important in an interfacial polymerisation as it increases the interfacial area available for reaction and speeds up diffusion of reagents to and product from the interface through forced convection. Stirring, therefore, increases the availability of the diamine for reaction and previous workers have correlated increases in stirring with the equivalent amount of surfactant required to achieve similar rates of polyesterification[35,68]. If the stirring speed is increased to such an extent that the concentration of the diamine increases too much then the interface may become saturated with polymeric material and the rate of polymerisation can fall. The volume of a droplet dispersed within the continuous phase is proportional to the cube of its radius whilst the interfacial area is proportional to the square of the radius. As the size of the droplet is reduced the ratio of surface area to volume of the droplet is increased. Under most circumstances this has a beneficial effect on the reaction rate and, therefore, the molecular weight of the polymer produced. If, however, the droplet size is reduced too much by excessive stirring then all of the monomer within the droplet may be exhausted leaving an excess of the other monomer at the interface. Under such conditions a drop in the molecular weight would be expected since there is no longer a balance of reagents at the locus of polymerisation. An homogeniser provides high turbulent mixing efficiency even at low speed.

The number average molecular weight drops significantly whilst a progressive increase in the weight average with increased stirring speed is observed (Graph 5.7 and Graph 5.8). The polydispersity is higher at faster stirring speeds (Graph 5.9). The number average molecular weight shows a greater dependence on lower molecular weight material whilst the weight average molecular weight is more influenced by higher molecular weight material. Increasing the stirring speed increases contact between the diamine and diacyl chloride, resulting in an increase in the weight average molecular weight. Increased contact between the diacyl chloride and the basic aqueous phase would increase hydrolysis in the latter stages of the reaction when the concentration of diamine was low. This would explain the fall in the number average molecular weight.

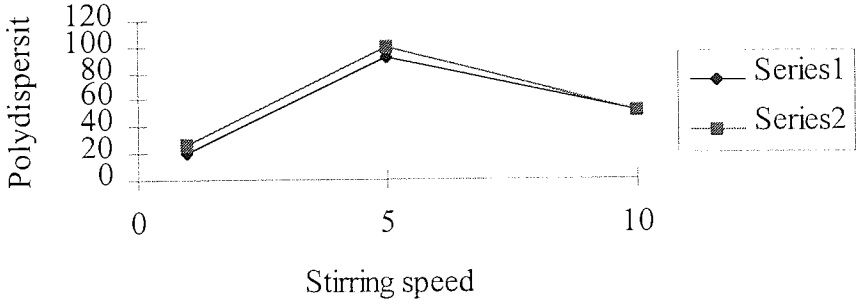
Graph 5.4 Variation in Mw of interfacially synthesised poly (ornithine *iso* -phthalamide) with stirring speed.



Graph 5.5 Variation in Mn of interfacially synthesised poly (ornithine *iso* -phthalamide) with stirring speed.



Graph 5.6 Variation in polydispersity of interfacially synthesized poly (ornithine *iso* - phthalamide) with stirring speed.



5.3.3 Stirring efficiency (ME 4.11.7-ME 4.11.10).

Increasing the stirring speed does not necessarily increase the stirring efficiency of the system. For example in a vortexing system the stirring efficiency is minimal. Laminar flow of liquid of equal density occurs and can result in an ordering of dispersed solid particles according to size. Increasing the stirring speed will just increase the speed of rotation of the vortex. Introduction of baffles in the reaction vessels can disrupt such laminar flow inducing radial or vertical flow streams depending on the type of impeller. The homogeniser provides an integral baffling system in the form of a dispersion generator which generates an emulsion by interaction with the liquid stream flowing from the dispersion head, a two or four pronged piece of metal that rotates within the dispersion generator. The stirring efficiency of the homogeniser was modified by varying the combination of dispersion head and generator according to section 4.

The nature of the dispersion generator and homogenising head significantly effect the molecular weight averages of the polymer (Table 5.2). The ultra fine dispersion generator gave a product with an increased molecular weight whilst the ultra fine homogenising head caused a reduction in the molecular weight. The ultra fine dispersion generator clearly improved the stirring efficiency of the system whereas the ultra fine homogenising head somehow reduced the stirring efficiency.

A reduction, rather than an excessive increase, in stirring efficiency is indicated by the fact that the combination of ultrafine homogenising head and ultrafine dispersion generator gave a higher molecular weight than the ultrafine homogenising head and fine dispersion generator. Increasing the stirring efficiency by replacing the fine dispersion generator with the ultrafine dispersion generator would be expected to give a further reduction in the molecular weight if the stirring efficiency was already excessive. The four pronged homogenising head may induce less mass flow of liquid through the dispersion generator than the two pronged generator. This would explain the decrease in molecular weight observed on replacing the fine head with the ultra fine head in combination with the fine dispersion generator and the increase in molecular weight when the fine dispersion generator was placed with the ultra fine dispersion generator.

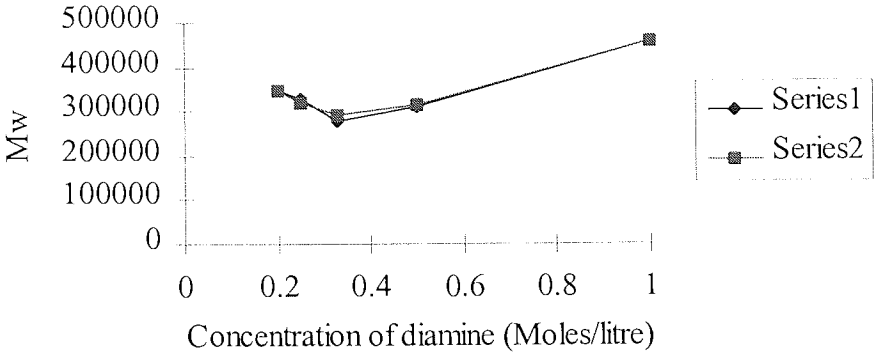
Table 5.2 Variation of molecular weight averages and polydispersisities of interfacially synthesised poly (ornithine *iso*-phthalamide) with variation in the stirring efficiency.

Samples Analysed 22.7.93 Solvent: DMF with no LiBr			
Experimental variation from general method	Mw 1st run 2nd run	Mn 1st run 2nd run	Mw/Mn 1st run 2nd run
Ultrafine head / Ultrafine generator ME 4.11.10	405000 411000	7930 8290	51.0 49.6
Ultrafine head / Fine generator ME 4.11.8	340000 340000	7570 7180	44.9 47.3
Fine head / Ultrafine generator ME 4.11.9	434000 436000	9270 9610	46.8 45.4
Fine head / Fine generator ME 4.11.7	371000 374000	7910 7130	46.9 52.5

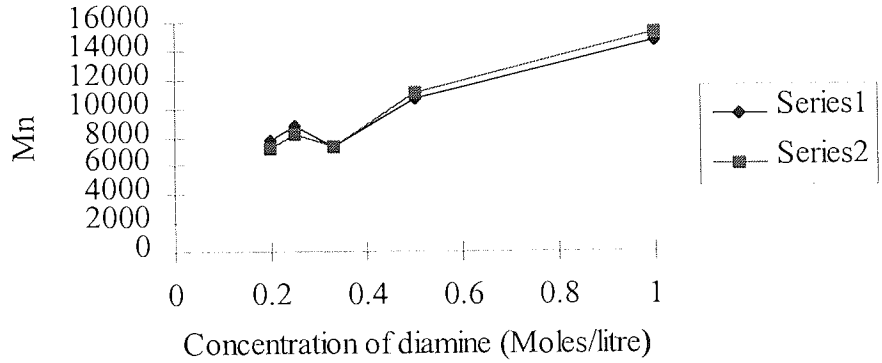
5.3.4 Concentration of the aqueous phase (ME 4.11.11-ME 4.11.15).

The concentration of the diamine in the aqueous phase will effect the equilibrium partition coefficient of the diamine in the organic phase. As the concentration of the aqueous phase was increased the molecular weight averages initially fell and then rose again (graphs 5.7 and 5.8). As the concentration of the diamine is increased the equilibrium partition coefficient in the organic solvent will increase and thus the availability of the diamine for reaction will increase. On the other hand, as the volume of one of the phases is reduced so the stirring efficiency and the amount of interfacial area generated is also reduced. This will oppose any increase in molecular weight because of increased availability of the diamine. Eventually the increase in diamine concentration outweighs the effects of stirring. The polydispersity falls with increasing diamine concentration despite the initial fall in molecular weight (Graph 5.9).

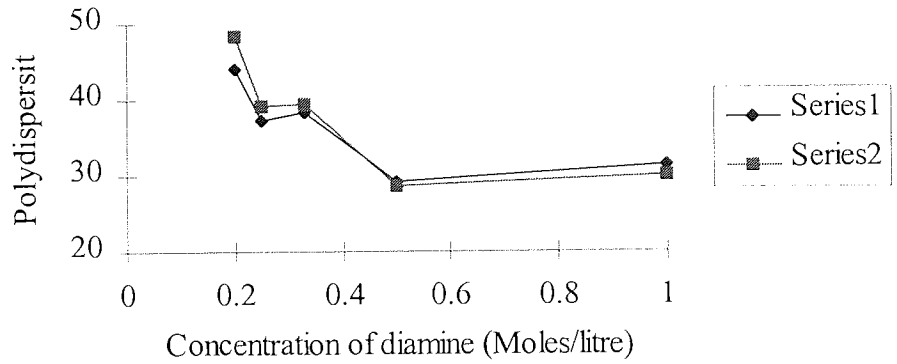
Graph 5.7 Variation in Mw of interfacially synthesised poly (ornithine *iso*- phthalamide) with concentration of diamine.



Graph 5.8 Variation in Mn of interfacially synthesised poly (ornithine *iso*- phthalamide) with concentration of diamine.



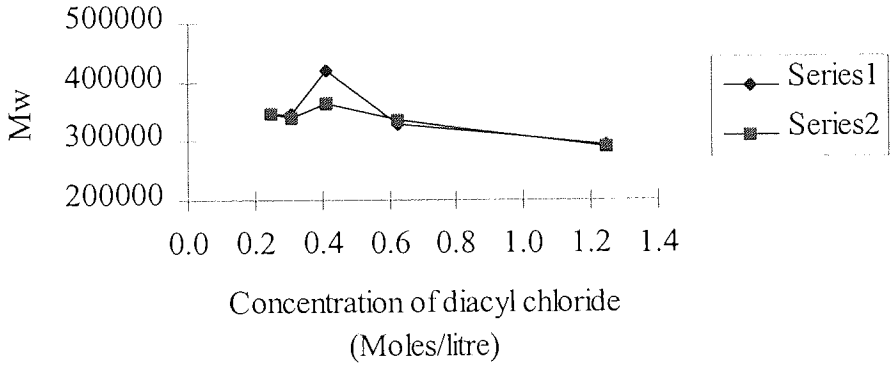
Graph 5.9 Variation in polydispersity of interfacially synthesised poly (ornithine *iso*- phthalamide) with concentration of diamine.



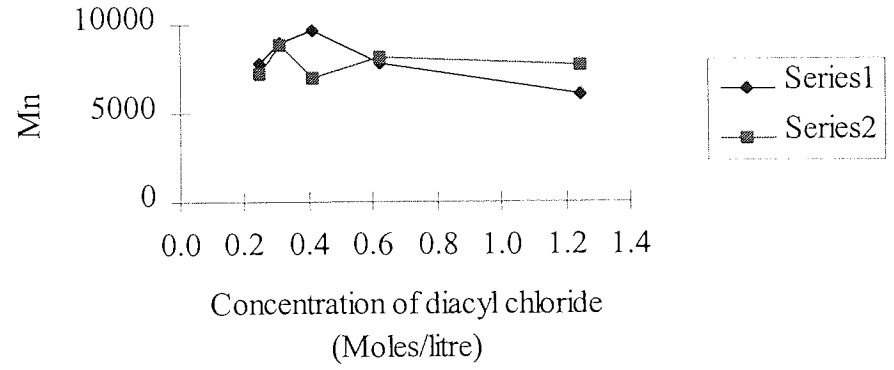
5.3.5 Concentration of the organic phase (ME 4.11.16-ME 4.11.20).

Increasing the concentration of the organic phase will increase the equilibrium partition coefficient of the diacyl chloride in the aqueous phase and may cause a change in the locus of polymerisation. The molecular weight of the polymer was increased by increasing the concentration of the organic phase (graphs 5.10 and 5.11). The increased availability of the diacyl chloride must outweigh the decrease in stirring efficiency. Eventually the molecular weight began to drop either because of further decreases in stirring efficiency or due to a saturation of the locus of polymerisation with diacyl chloride. The polydispersity decreased initially then rose slightly with increasing diacyl chloride concentration (Graph 5.12).

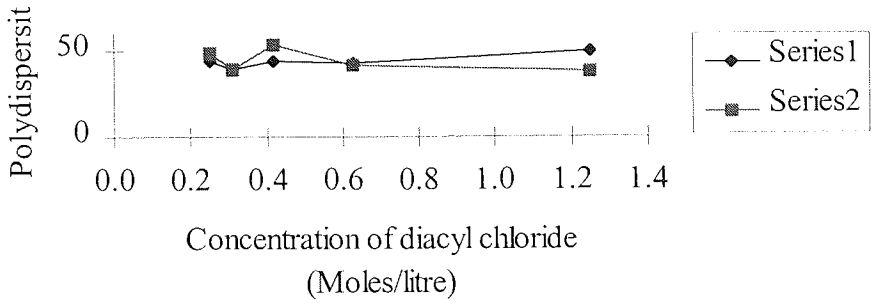
Graph 5.10 Variation of Mw of interfacially synthesised poly (ornithine *iso*- phthalamide) with concentration of diacyl chloride.



Graph 5.11 Variation in Mn of interfacially synthesised poly (ornithine *iso*- phthalamide) with concentration of diacyl chloride.



Graph 5.12 Variation in polydispersity of interfacially synthesised poly (ornithine *iso*-phthalamide) with concentration of diacyl chloride.

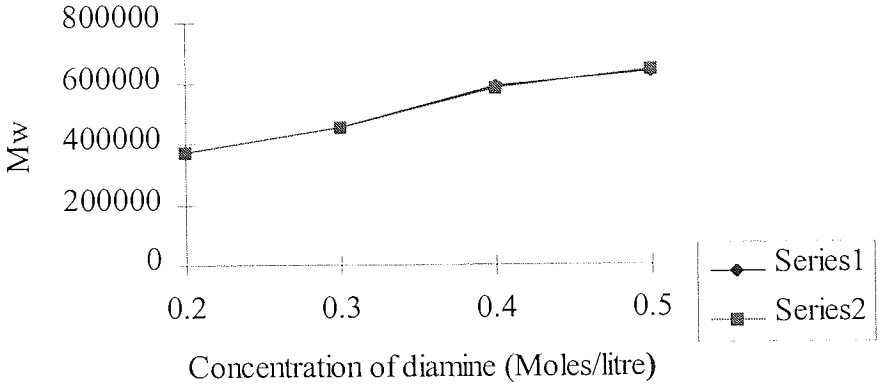


5.3.6 Stoichiometry of the organic and aqueous phase (ME 4.11.21-ME 4.11.26).

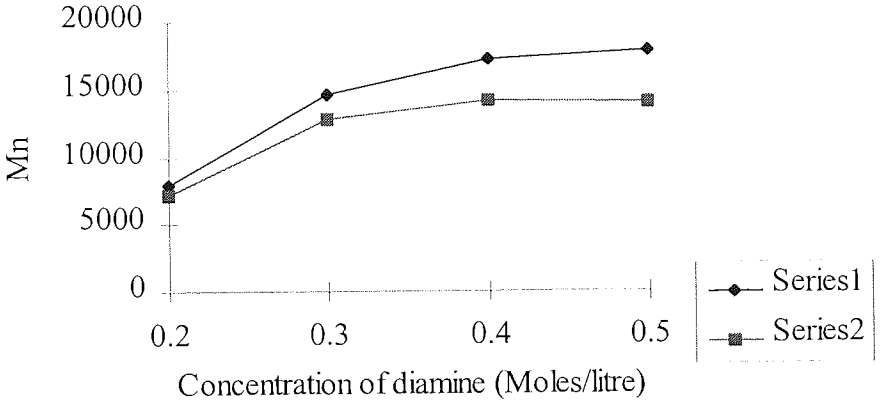
The availability of the diamine can be increased independently of the phase volume by increasing the concentration beyond the stoichiometric amount. Stirring efficiency should not be effected (except by changes in the solution viscosity) and so a clearer picture of the requirements of diamine availability should be obtained.

As the concentration of the diamine is increased (constant volume) the molecular weight increases (graphs 5.13 and 5.14) as does the polydispersity (Graph 5.15). The increase in molecular weight is attributed to the increase in availability of the diamine at the locus of polymerisation. A similar increase in the molecular weight and polydispersity of the polymer was seen when the concentration of the diacyl chloride was doubled (Table 5.3). Increases in the molecular weight, therefore, indicate that increasing the availability of either reagent is beneficial to the reaction. Increasing the tonicity of the aqueous phase would reduce the solubility of the oligomers within the bulk of the aqueous phase and thus reduce their removal from the locus of polymerisation. The increases in polydispersity result from the formation of lower molecular weight material towards the end of the reaction. At the initial stages of the reaction there is a balance of reagents provided by diffusion at the interface, whereas the relative excess of one reagent becomes increasingly large at the end of the reaction and polymer formation takes place under increasingly unfavourable conditions.

Graph 5.13 Variation in Mw of interfacially synthesised poly (ornithine *iso*- phthalamide) with excess diamine.



Graph 5.14 Variation in Mn of interfacially synthesised poly (ornithine *iso*- phthalamide) with excess diamine.



Graph 5.15 Variation in polydispersity of interfacially synthesised poly (ornithine *iso*- phthalamide) with excess diamine.

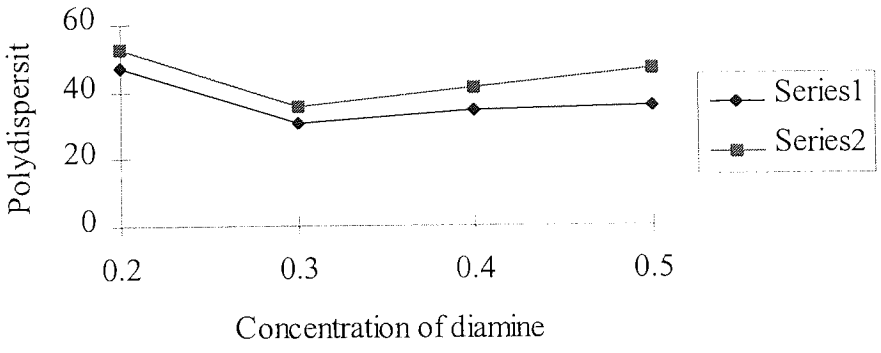
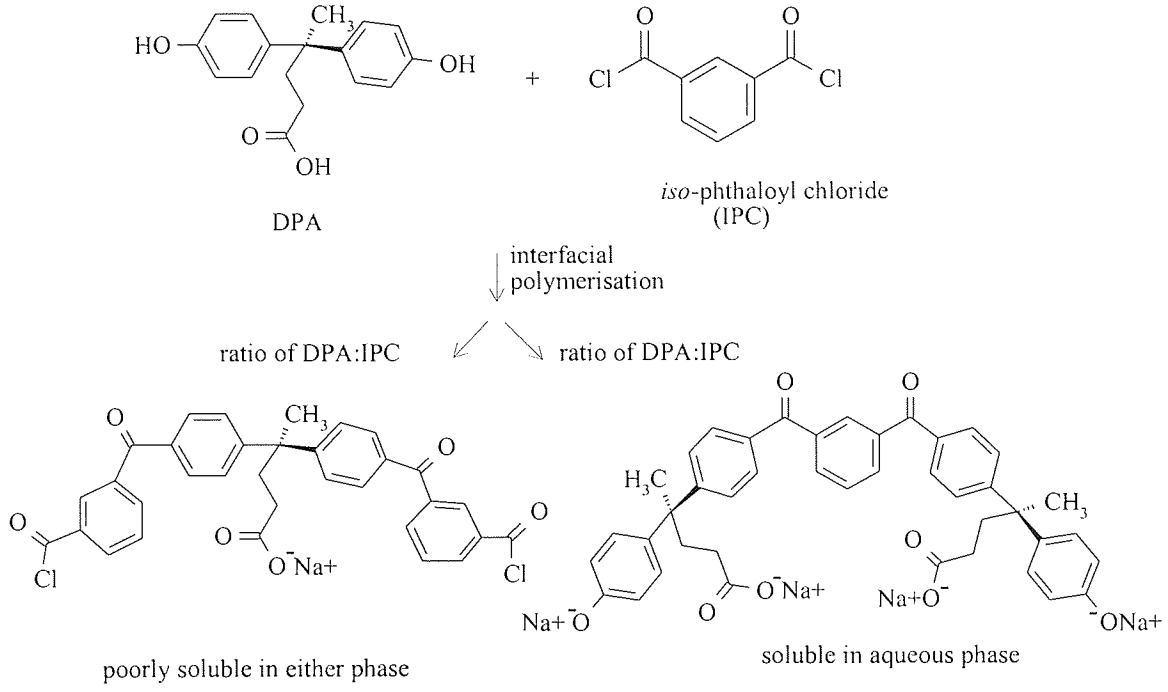


Table 5.3 Variation of molecular weight averages and polydispersisities of interfacially synthesised poly (ornithine *iso*-phthalamide) with excess diacyl chloride.

Samples Analysed 22.7.93 Solvent: DMF with no LiBr			
Experimental variation from general method	Mw 1st run 2nd run	Mn 1st run 2nd run	Mw/Mn 1st run 2nd run
5mL 0.5M *iso*-phthaloyl chloride ME 4.11.26	542000 544000	12500 12000	43.5 45.3
5mL 0.25M *iso*-phthaloyl chloride ME 4.11.21	222000 220000	10800 8460	20.6 26.0

The fact that increases in both monomer concentrations caused an increase in molecular weight suggests that the locus of polymerisation may change depending on the relative concentrations of the diamine and diacyl chloride since if the locus of polymerisation was the organic side of the interface, then why, in view of a limited supply of diamine, should increasing the diacyl chloride concentration benefit the reaction? Wang *et. al.*[27] synthesised functional aromatic polyesters bearing pendant carboxyl groups on the side chain from *iso*-phthaloyl chloride and 4,4'-bis-(p-hydroxyphenyl) valeric acid (DPA) (Fig. 5.4). These authors found that the ratio of DPA to *iso*-phthaloyl chloride was critical in the formation of high molecular weight polymer and proposed two mechanisms to account for the differences in molecular weight.

Fig. 5.4 Structure of oligomers formed in the initial stages of reaction between iso-phthaloyl chloride and 4,4'-bis-(p-hydroxyphenyl) valeric acid and their effect on solubility.



The structure of the oligomers formed at the initial stages of the reaction effects the solubility of the oligomers. In a typical interfacial polyesterification the ratio of bis-phenol to diacyl chloride may be greater than 1. The oligomers formed in such a situation would have phenolate end groups and are found to be insoluble in both the organic and aqueous phases. However, when DPA was used as the bis-phenol the reduced viscosities of the polymers produced with ratios of DPA to diacyl chloride greater than 1 (1.20 and 1.15) were found to be substantially lower than if the molar ration of DPA to diacyl chloride was less than 1. The lower reduced viscosities of the polymers were attributed to the abstraction of phenolate ended oligomers bearing pendant carboxylate groups into the aqueous phase. Polymerisation must then occur predominantly on the aqueous side of the interface. When the molar ratio of DPA to diacyl chloride is less than 1 (0.91 and 0.83) the oligomers having carboxylate groups in the side chains have acyl chloride end groups and so are insoluble in both phases.

Polyester with reasonably high molecular weight was obtained with a molar ratio of DPA to diacyl chloride of 1.05 since the oligomers were only partially soluble in the aqueous phase. Therefore, by altering the molar ratio of diamine to diacyl chloride the locus of polymerisation is changed. Why then do the molecular weights of the polyamides increase when the polyesters show a decrease in molecular weight. The rates of reaction between bis-phenolates and diacyl chlorides are lower than those between diamines and diacyl chlorides. The polymerisations of Wang *et. al.* lasted 3 hrs and it was demonstrated that the yields and molecular weights of polymers deliberately precipitated from reaction after 15 minutes were much lower than those obtained after 3 hrs. The lower rate of reaction greatly increases the importance of competing hydrolysis. In the synthesis of poly (ornithine *iso*-phthalamide) the reaction was essentially complete after 10 minutes. Moving the locus of polymerisation from the organic to the aqueous side of the interface increases contact between the amine functions and the acid chloride terminated oligomers. The subsequent rapid reaction out paces hydrolysis of the aromatic acid chloride.

5.3.7 Organic solvent (ME 4.11.27-ME 4.11.29).

The nature of the organic solvent plays an important role in an interfacial polymerisation (Table 5.4). Solvent polarity and partial miscibility will effect the transfer of reagents across the interface and will effect the equilibrium partition coefficients of the diacyl chloride and diamine in the aqueous and organic phases. Morgan[1] determined the amount of organic solvent in the aqueous phase and *vice versa* for a number of solvent systems commonly used in interfacial polycondensations (Table 5.5). The trends in molecular weight follow the phase miscibilities of the organic and aqueous phases. In polycondensations where access of one of the reagents to the locus of polymerisation is limited because of a low partition coefficient, then partial phase miscibility would be beneficial. Clearly, as the phase miscibility is increased then contact between the reagents will be increased. Increased phase miscibility leads to a less well defined interface which may explain the increases in polydispersity with increasing phase miscibility.

Table 5.4 Variation of molecular weight averages and polydispersisities of interfacially synthesised poly (ornithine *iso*-phthalamide) with the nature of the organic solvent.

Samples Analysed 22.7.93 Solvent: DMF with no LiBr			
Experimental variation from general method	Mw 1st run 2nd run	Mn 1st run 2nd run	Mw/Mn 1st run 2nd run
Carbon tetrachloride ME 4.11.21	222000 220000	10800 8460	20.6 26.0
Hexane ME 4.11.28	340000 337000	11300 10800	30.2 31.3
Toluene ME 4.11.27	383000 372000	11400 12000	33.7 30.9
chloroform ME 4.11.29	473000 460000	12900 14200	36.6 32.4

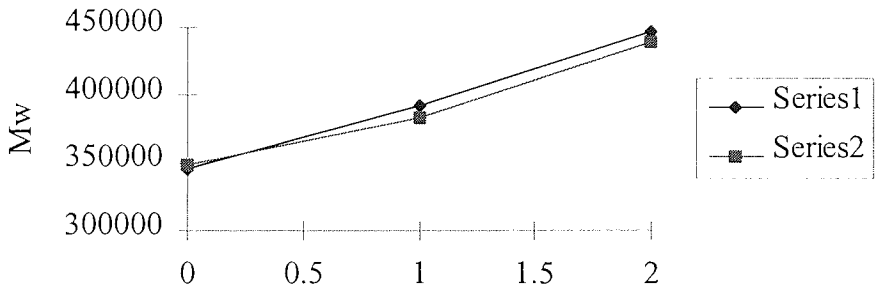
Table 5.5 Phase miscibility and interfacial tension of common solvent systems in interfacial polycondensation.

Organic solvent	Water in solvent (g/100g)	Solvent in water (g/100g)	Interfacial tension (Dynes/cm at 20°C)
CCl_4	0.0100	0.0020	45
Hexane	0.0111	0.0140	51.1
Toluene	0.0500	0.0630	36.1 (25°C)
chloroform	0.0700	0.7800	32.8

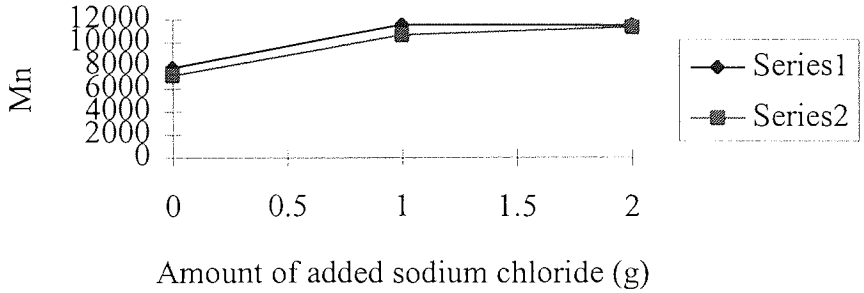
5.3.8 Added salt (ME 4.11.30-ME 4.11.32).

Addition of sodium chloride to the aqueous phase increases the molecular weight and polydispersity of the polymer (graphs 5.16-5.18). The increase in molecular weight is due to salting out of the ornithine. The increase in polydispersity could be due to reduced diffusion of polymer from the interface, which would effectively slow down the reaction at high conversion by clogging the interface with polymer and reducing the diffusion of reactants to the locus of polymerisation.

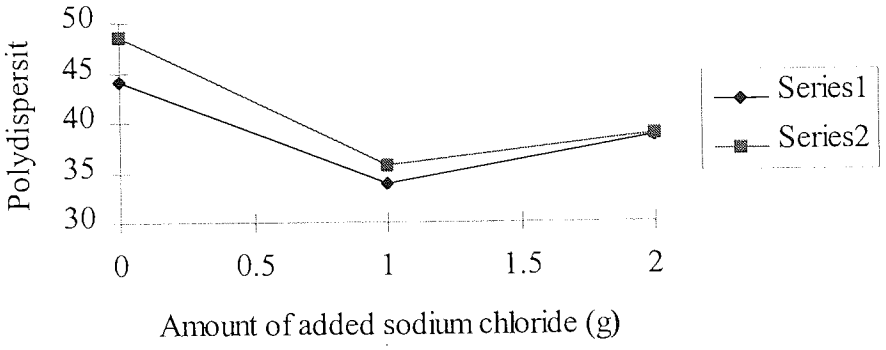
Graph 5.16 Variation in Mw of interfacially synthesised poly (ornithine *iso*-phthalamide) with amount of added sodium chloride.



Graph 5.17 Variation in Mn of interfacially synthesised poly (ornithine *iso*-phthalamide) with amount of added sodium chloride.



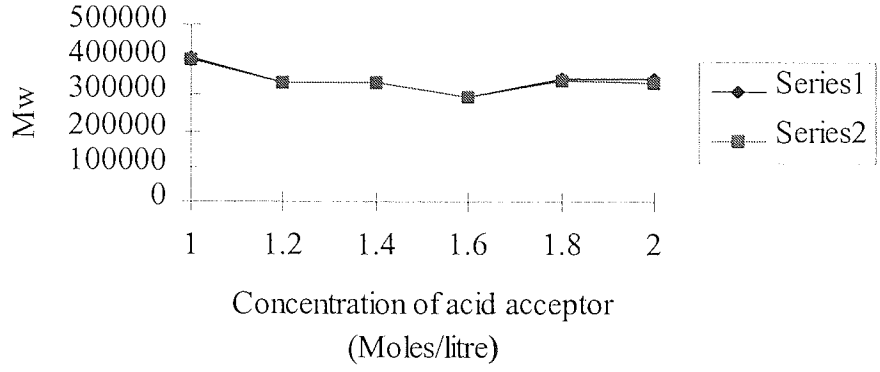
Graph 5.18 Variation in polydispersity of interfacially synthesised poly (ornithine *iso*-phthalamide) with amount of added sodium chloride.



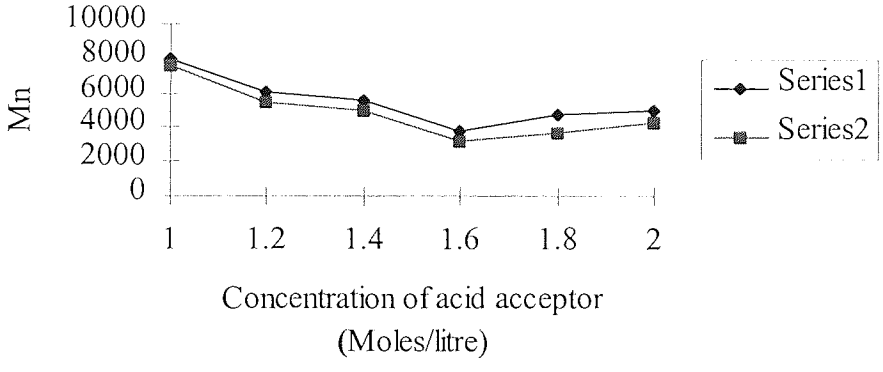
5.3.9 Concentration of the acid acceptor (ME 4.11.33-ME 4.11.40).

The concentration of acid acceptor determines the availability of diamine in a reactive form in the aqueous phase. Balanced with the need for the diamine to be in the free base form is the problem of hydrolysis of the acid chloride. As the concentration of acid chloride is increased from 1.0M there is an initial decrease in molecular weight of the polymer (Graph 5.19 and 5.20), this decrease is attributed to the increased hydrolysis of the diacyl chloride. As the concentration of the acid acceptor is further increased, the molecular weight begins to rise again. This unexpected effect may be due to a salting out effect, which as seen in Sec. 5.3.7 caused an increase in the molecular weight due to increased availability of the diamine. The polydispersity decreased with increasing molecular weight (Graph 5.21).

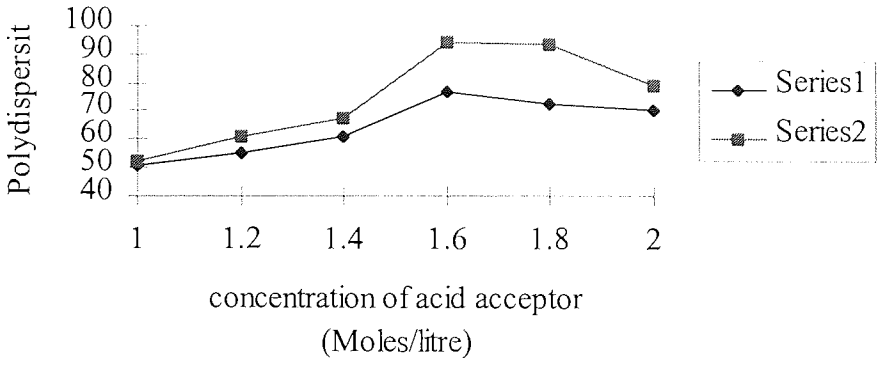
Graph 5.19 Variation in Mw of interfacially synthesised poly (ornithine *iso*- phthalamide) with concentration of acid acceptor.



Graph 5.20 Variation in Mn of interfacially synthesized poly (ornithine *iso*- phthalamide) with concentration of acid acceptor.



Graph 5.21 Variation in polydispersity of interfacially synthesized poly (ornithine *iso*- phthalamide) with concentration of acid acceptor.



5.3.10 Nature of the acid acceptor (ME 4.11.41-ME 4.11.42).

The molecular weight of poly (ornithine *iso*-phthalamide) is dependent on the nature of the acid acceptor (Table 5.6). Sodium carbonate is a weaker base than sodium hydroxide and so the increase in molecular weight seen when sodium carbonate was used as the acid acceptor was attributed to the lower hydrolysis of the diacyl chloride. Triethylamine is also a weak base and so hydrolysis may be expected to be lower. Morgan[1], however, states that organic bases interact unfavourably with the acyl chlorides in interfacial synthesis and Wang *et. al.*[27] state that triethylamine is not suitable for the water/organic phase interfacial polycondensation reaction because of a catalysing effect of the tertiary amine on the acid chloride. The complex formed between the acid chloride and the tertiary amine shows enhanced reactivity of the acyl group due to an increase in the partial positive charge on the carbonyl carbon. The enhanced electrophilicity of the carbonyl carbon facilitates nucleophilic attack not only by the bis-phenolate, but by water and the carboxylate pendant functions of the bis-phenol. Characteristic absorption bands of anhydride groups were found in the infra red spectra of the products. Contrary to these findings, triethylamine gave improved molecular weights in interfacially synthesised poly (ornithine *iso*-phthalamide) relative to sodium hydroxide and no evidence of anhydride formation was found. The differences between the two systems are attributed to the higher nucleophilicity of the amine functions of ornithine compared to the bis-phenolate.

When the concentration of the acid acceptor is lower than the concentration of hydrochloric acid generated throughout the reaction the diamine becomes the acid acceptor. Hydrolysis would be lower since the diamine is a weak base. The overall yield of the reaction would be reduced since the availability of the diamine in an active form would be reduced. It can be seen from the reaction where 0.2M sodium hydroxide was used as the acid acceptor that high molecular weight polymer can be produced when diamine is the acid acceptor.

Table 5.6 Summary of molecular weight averages and polydispersisities of interfacially synthesised poly (ornithine *iso*-phthalamide) with variation in the acid acceptor.

Samples Analysed 22.7.93 Solvent: DMF with no LiBr			
Experimental variation from general method	Mw 1st run 2nd run	Mn 1st run 2nd run	Mw/Mn 1st run 2nd run
0.2M sodium hydroxide ME 4.11.40	431000 445000	9150 8200	47.0 54.3
0.8M sodium carbonate ME 4.11.41	631000 634000	23600 21900	26.8 28.9
0.8M triethylamine ME 4.11.42	541000 544000	13700 13200	39.4 41.2

5.4 EFFECT OF REACTION CONDITIONS ON THE MOLECULAR WEIGHT AVERAGES AND POLYDISPERSISITIES OF POLY (LYSINE ETHYL ESTER *ISO*-PHTHALAMIDE) SYNTHESISED BY THE MISCIBLE MIXED SOLVENT METHOD.

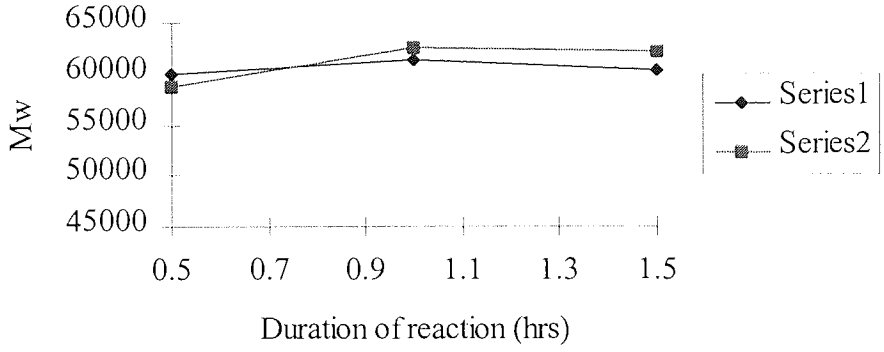
The synthesis of poly (lysine ethyl ester *iso*-phthalamide) using a miscible mixed solvent system under various experimental conditions is described in Sec. 3.3. The number and weight average molecular weights and polydispersisities of the samples thus prepared are presented and discussed with relation to the experimental conditions employed.

The molecular weight averages and polydispersisities are expressed as PEO/PEG equivalents and were obtained by G.P.C. using DMF containing 0.01%. lithium bromide as the mobile phase.

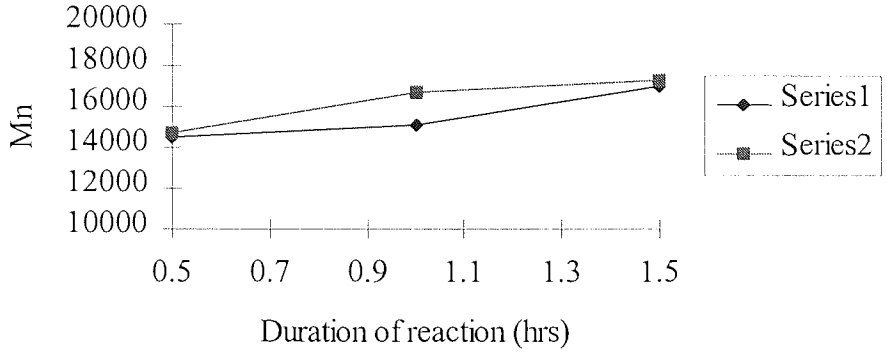
5.4.1 Reaction time (ME 3.3.1-ME 3.3.3).

The number and weight average molecular weights rise slightly with increasing reaction time (graphs 5.22 and 5.23) with a consequent decrease in the polydispersity of the polymer (Graph 5.24). It can be seen that the weight average molecular weight falls slightly on increasing the reaction time from 1hr to 1 1/2 hrs. The variations are relatively small and may be the result of stirring inconsistencies. Evaporation of the acetone component of the solvent system will result in a reduction in the solubility of the lower molecular weight oligomers retained in solution after precipitation of the bulk of the polymer. The precipitation of low molecular weight oligomers would be expected to have a greater effect on the number average molecular weight than the weight average molecular weight and would also cause an increase in the polydispersity of the sample rather than the observed decrease. It is likely that the relatively small changes in molecular weight seen are due to minor variations in experimental conditions rather than as a result of increasing reaction time and that the reaction is essentially complete after 30 minutes

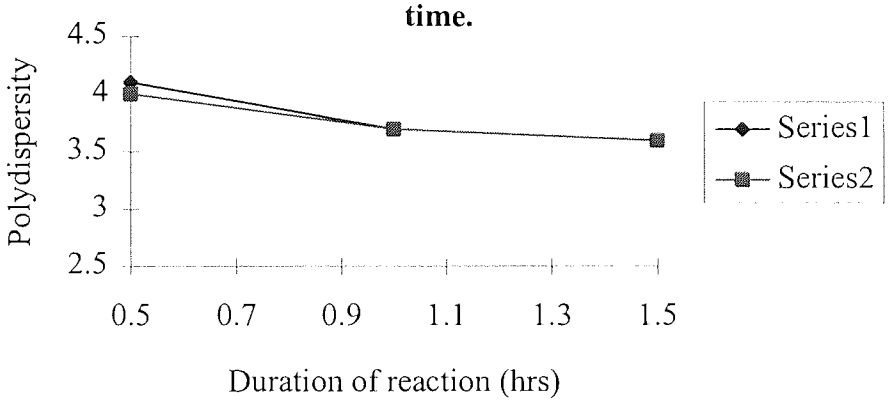
Graph 5.22 Variation in Mw of poly (lysine ethyl ester *iso*- phthalamide) synthesised by the mixed miscible solvent method with reaction time.



Graph 5.23 Variation in Mn of poly (lysine ethyl ester *iso*-phthalamide) synthesised by the mixed miscible solvent method with reaction time.



Graph 5.24 Variation in polydispersity of poly (lysine ethyl ester *iso*-phthalamide) synthesised by the mixed miscible solvent method with reaction time.

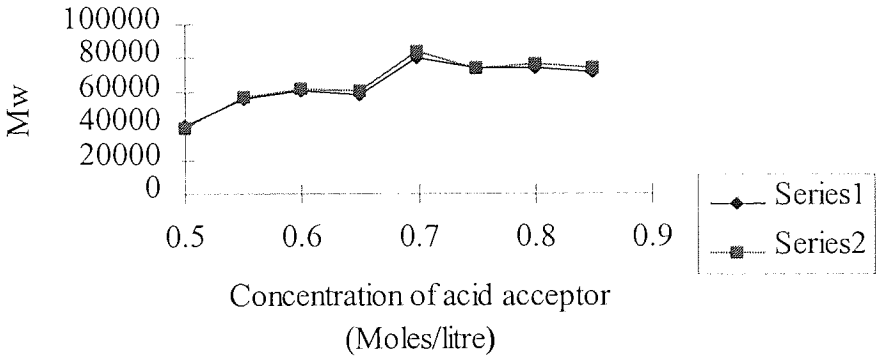


5.4.2 Concentration of acid acceptor (ME 3.2.1-ME 3.2.8).

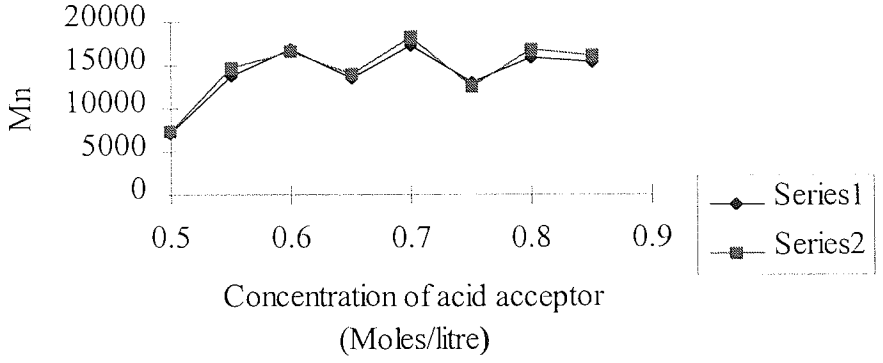
Both number average and weight average molecular weights increase with the concentration of acid acceptor to a maximum value and then begin to fall with further increases (Graphs 5.25 and 5.26). Deviations in this trend occur at acid acceptor concentrations between 0.65 and 0.75M due to differences in the stirring conditions employed.

A combination of shorter magnetic follower and a different stirrer/hot plate caused lower stirring efficiency resulting in a reduction of the molecular weight. The stoichiometric concentration of acid acceptor would be 0.8M, since at a monomer concentration of 0.2M, the concentration of HCl generated by complete reaction of the diacyl chloride would be 0.4M and that generated on liberation of the free diamine from the dihydrochloride salt of lysine ethyl ester. 2HCl would also be 0.4M. At a concentration of less than 0.8M potassium hydrogen carbonate must partially act as the acid acceptor. Hydrogen carbonates are regarded by some workers as insufficiently basic to function adequately as the acid acceptor in interfacial polycondensations. This is supported by the decrease in molecular weight as the proportion of HCl that must be neutralised by hydrogen carbonate increases.

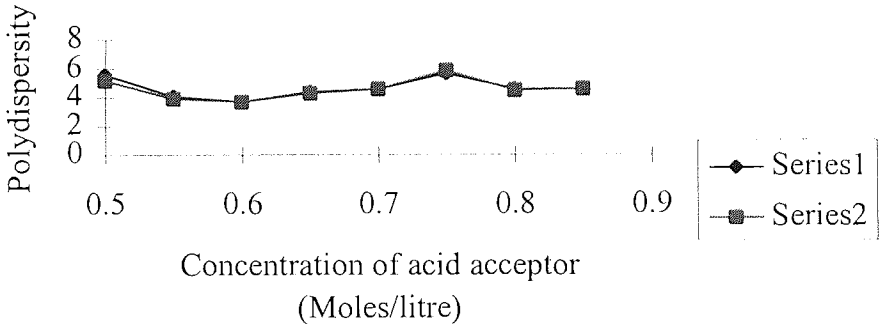
Graph 5.25 Variation in Mw of poly (lysine ethyl ester *iso*- phthalamide) synthesised by the miscible mixed solvent method with concentration of acid acceptor.



Graph 5.26 Variation in Mn of poly (lysine ethyl ester *iso*- phthalamide) synthesised by the miscible mixed solvent method with concentration of acid acceptor.



Graph 5.27 Variation in polydispersity of poly (lysine ethyl ester *iso*- phthalamide) with concentration of acid acceptor synthesised using the miscible mixed solvent method.



Above the stoichiometric amount of potassium carbonate the molecular weight began to fall due to increased hydrolysis of the diacyl chloride in an increasingly basic environment. Since there is only one phase the diacyl chloride is afforded no protection from hydrolysis by retention in an organic solvent that is immiscible with the aqueous phase. The polydispersity initially falls as expected with increasing concentration of acid chloride acceptor and the resulting increase in molecular weight (Graph 5.27).

As the concentration of acid acceptor increases further the polydispersity begins to rise again before the maximum molecular weight is reached. This effect is most probably due to an increase in hydrolysis at higher acid acceptor concentrations causing a broadening of the molecular weight distribution.

Analysis of the film formed over night at the air/liquid interface showed that the material was low molecular weight with a narrow polydispersity (Table 5.7)

Table 5.7. Summary of molecular weight averages and polydispersisities of poly (lysine ethyl ester *iso*-phthalamide) precipitated as a film at the air/liquid interface overnight.

Sample Analysed 26.4.95 in DMF with 100ppm LiBr			
Experimental variation from general method	Mw 1st run 2nd run	Mn 1st run 2nd run	Mw/Mn 1st run 2nd run
Overnight precipitate from mixed miscible solvent reaction General method 2 0.5M K_2CO_3 ME 3.6.2	2510 2570	1070 1130	2.4 2.3

5.4.3 Time of addition of second phase (ME 37.1-ME 3.7.4.1.).

The timing and order of addition of the two miscible phases is critical in the production of high molecular weight polymer by this method. Rapid addition of either phase to the other resulted in high molecular weight polymer as did slow addition of the diacyl chloride to the basic diamine solution. However, slow addition of the diamine to the diacyl chloride solution gave a product with a much reduced molecular weight (Table 5.8).

When the basic diamine solution is added slowly to the diacyl chloride solution the diacyl chloride will initially be present in excess and so the amine functions will be acylated to a large extent. The availability of the diamine is then essentially reduced allowing competing hydrolysis to occur. Slow addition of the diacyl chloride solution to the basic diamine solution means that the diamine is initially present in excess. Reaction with the excess of diamine results in amine ended trimers which condense as more diacyl chloride is added. Hydrolysis is not a problem since the diacyl chloride reacts rapidly with the diamine and is never present in excess. The higher number average molecular weight for the sample prepared by rapid addition of diamine to diacid may be due to the higher proportion of acetone in the solvent mixture at the initial stages of the reaction. The precipitated polymer is swollen by the acetone/water mixture and higher proportions of acetone may increase the mobility of the polymer chains in the initial stages of the reaction allowing a higher degree of polymerisation before precipitation. Polymerisation would continue to a greater extent after precipitation if the precipitate was considerable more swollen by the solvent. This method of addition gives the narrowest polydispersity of the high molecular weight samples.

The slightly higher weight average molecular weight of the sample prepared by the slow addition of the diacyl chloride solution to the diamine solution could result from more efficient mixing of the oligomers and monomers in the initial stages of the reaction because of the lower rate of reaction. Towards the end of the reaction the relative excess of diamine would be reduced so that polycondensation becomes increasingly less favourable. This sample had the highest polydispersity of the higher molecular weight samples. Similar trends were seen in the polycondensation of lysine methyl ester with *iso*-phthaloyl chloride under similar conditions (Table 5.9).

Table 5.8 Molecular weight averages and polydispersisities of poly (lysine ethyl ester *iso*-phthalamide) synthesised by the mixed miscible solvent method with variation in the method of reactant addition.

Samples Analysed 16.5.95 in DMF with 100ppm LiBr			
Experimental variation from general method	Mw 1st run 2nd run	Mn 1st run 2nd run	Mw/Mn 1st run 2nd run
Fast addition of diacyl chloride solution ME 3.7.1	56000 56300	14800 15200	3.8 3.7
Fast addition of diamine solution ME 3.7.2	55400 53900	17500 17400	3.2 3.1
Slow addition of diacyl chloride solution ME 3.7.3	65900 66100	15100 15100	4.4 4.4
Slow addition of diamine solution ME 3.7.4.1	16000 15600	5640 5590	2.8 2.8

Table 5.9 Molecular weight averages and polydispersisities of poly (lysine methyl ester *iso*-phthalamide) synthesised by the mixed miscible solvent method with variation in the method of reactant addition.

Samples Analysed 26.4.95 in DMF with 100ppm LiBr			
Experimental variation from general method	Mw 1st run 2nd run	Mn 1st run 2nd run	Mw/Mn 1st run 2nd run
Fast addition of diacyl chloride solution ME 3.8.1	37700 38100	5960 5410	6.3 7.0
Fast addition of diamine solution ME 3.8.2	41000 40200	8700 7360	4.7 4.2
Slow addition of diamine chloride solution ME 3.8.2	11400 11900	2420 2830	4.7 4.5

5.4.4 Stirring efficiency.

Stirring efficiency had a significant effect on the molecular weight of polyamides synthesised by the mixed miscible solvent technique (Table 5.10). A decrease in molecular weight was seen on scaling up the reaction from a total liquid volume of 0.1 to 1 litre. The decrease in weight average molecular weight indicated a lower mixing efficiency with the over head stirrer compared to a magnetic stirrer. Although the overhead stirrer would provide better mixing than a magnetic stirrer in an equivalent reaction vessel the increase in the scale of the reaction and, therefore, volume of liquid to mix may have resulted in a net lowering of the stirring efficiency. The mixing efficiency was more consistent with the overhead stirrer than when a magnetic stirrer was used since in the latter case polymer coagulating around the stirring bar caused disruption of stirring. When an overhead stirrer was used the precipitated polymer coagulated into a ball at the bottom of the reaction vessel allowing continued mixing of the liquid. This may explain the reduced polydispersity of the sample synthesised using an overhead stirrer A decrease in yield was associated with the decrease in molecular weight. This is consistent with solution polymerisations where maximum yield is attained under conditions where maximum molecular weight is achieved.

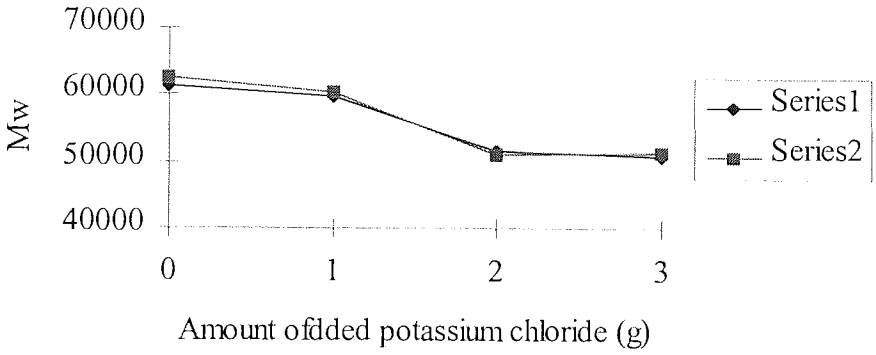
Table 5.10 Summary of molecular weight averages and polydispersisities of poly (lysine ethyl ester *iso*-phthalamide) synthesised by the mixed miscible solvent method with variation in stirring efficiency.

Samples Analysed 26.4.95 Solvent: DMF with 100ppm LiBr			
Experimental variation from general method	Mw 1st run 2nd run	Mn 1st run 2nd run	Mw/Mn 1st run 2nd run
Large stirring bar ME 3.6	74300 76500	16000 16900	4.6 4.5
Overhead stirrer ME 3.2	65900 65100	17800 18200	3.7 3.6

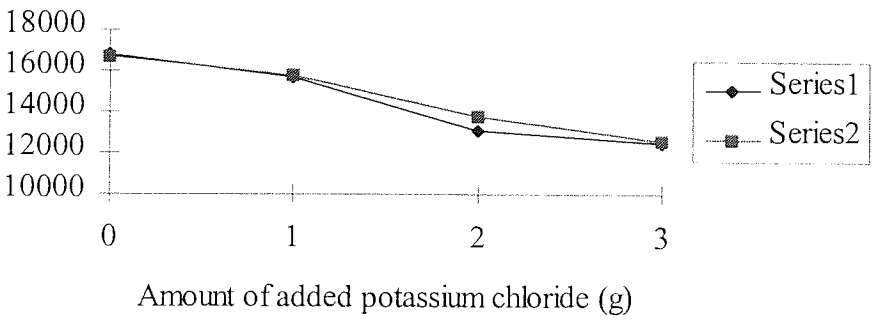
5.4.5 Added sodium chloride (ME 3.4.1-ME 3.4.3).

Addition of sodium chloride to the aqueous phase lowered the molecular weight of the polymer (graphs 5.28 and 5.29) and increased the polydispersity (Graph 5.30). The reduction in molecular weight is attributed to the reduced solubility of the polymer in the acetone/saline solvent system. This suggests that higher molecular weight polymer may be obtained by using the free base derivative of lysine ethyl ester. 2HCl. The free base derivatives are prone to cyclisation and self condensation[59] and should be prepared immediately prior to use.

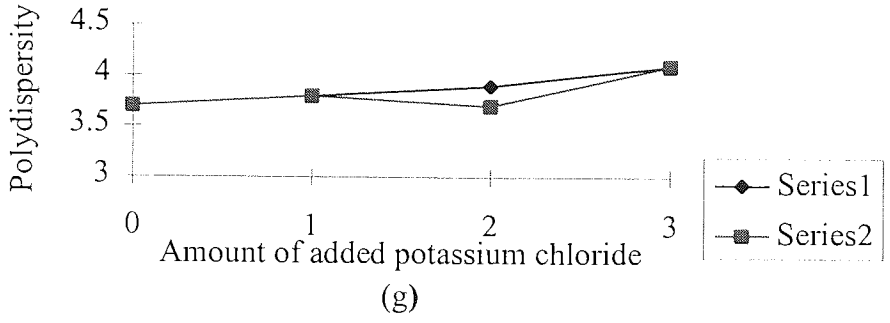
Graph 5.28 Variation in Mw of poly (lysine ethyl ester *iso*- phthalamide) synthesised by the miscible mixed solvent method with added potassium chloride .



Graph 5.29 Variation in Mn of poly (lysine ethyl ester *iso*- phthalamide) synthesised by the miscible mixed solvent method with added potassium chloride.



Graph 5.30 Variation in polydispersity of poly (lysine ethyl ester *iso*-phthalamide) synthesised by the miscible mixed solvent method with added potassium chloride.



5.4.6 Organic solvent (ME 3.5).

Changing the organic solvent from acetone to THF resulted in a reduction in molecular weight and an increase in the polydispersity(Table 5.11). This is attributed to the reduced solubility of the polymer in THF.

Table 5.11 Summary of molecular weight averages and polydispersisities of poly (lysine ethyl ester *iso*-phthalamide) synthesised by the mixed miscible solvent method with variation in the organic solvent.

Samples Analysed 26.4.95 Solvent: DMF with 100ppm LiBr			
Experimental variation from general method	Mw 1st run 2nd run	Mn 1st run 2nd run	Mw/Mn 1st run 2nd run
THF as organic solvent ME 3.5	18200 18500	2620 2780	7.0 6.7

5.4.7 Comparison of Molecular weight distributions of poly (lysine ethyl ester *iso*-phthalamide) synthesised by the interfacial and mixed miscible solvent methods.

Poly (lysine ethyl ester *iso*-phthalamide) synthesised by the miscible mixed solvent method had a higher molecular weight than a sample interfacially synthesised under similar conditions (Table 5.12). The process was, therefore, an acceptable alternative to the interfacial method for the synthesis of polyamides using diacyl chlorides that show resistance to hydrolysis. The lower polydispersity of the interfacially synthesised sample was due to the extraction of the crude polymer in refluxing acetone prior to analysis.

Extraction in refluxing acetone facilitated the removal of the organic solvent from the gelatinous precipitate and would have removed low oligomeric material. The molecular weight distributions of the two samples (Fig. 5.5) were similar in shape, whereas one would normally expect the interfacially synthesised sample to be more polydisperse with a bimodal shape as in the case of poly (lysine ethyl ester diethylmalonamide) (Sec5.6). The molecular weight distribution of a sample of poly (lysine ethyl ester *iso*-phthalamide) prepared interfacially under similar conditions with treatment in cold acetone showed a bimodal distribution (Fig. 5.6). The molecular weight distribution of the acetone extracts is multimodal, the highest molecular weight peak overlapping with the peak at low molecular weight on the extracted sample. The two lower molecular weight peaks in the molecular weight distribution of the acetone extracts may correspond to cyclic material and unreacted monomer. Unreacted diacyl chloride would have been hydrolysed to the diacid on removal of the acetone. No evidence of aromatic carboxylic acids was obtain from the FT-IR spectrum of the acetone extracts.

Table 5.12 Molecular weight averages and polydispersisities of poly (lysine ethyl ester *iso*-phthalamide) synthesised by the interfacial and miscible mixed solvent methods under similar conditions.

Samples Analysed 26.4.95 in DMF with 100ppm LiBr			
Experimental variation from general method	Mw 1st run 2nd run	Mn 1st run 2nd run	Mw/Mn 1st run 2nd run
Poly (lysine ethyl ester *iso*-phthalamide) synthesised by the miscible mixed solvent method	57500 57500	6550 7590	8.8 7.7
Poly (lysine ethyl ester *iso*-phthalamide) synthesised by the interfacial method	34800 34800	5860 5800	5.9 6.0

Fig. 5.5 Molecular weight distributions of poly (lysine ethyl ester iso-phthalamide) synthesised by the interfacial and miscible mixed solvent methods.

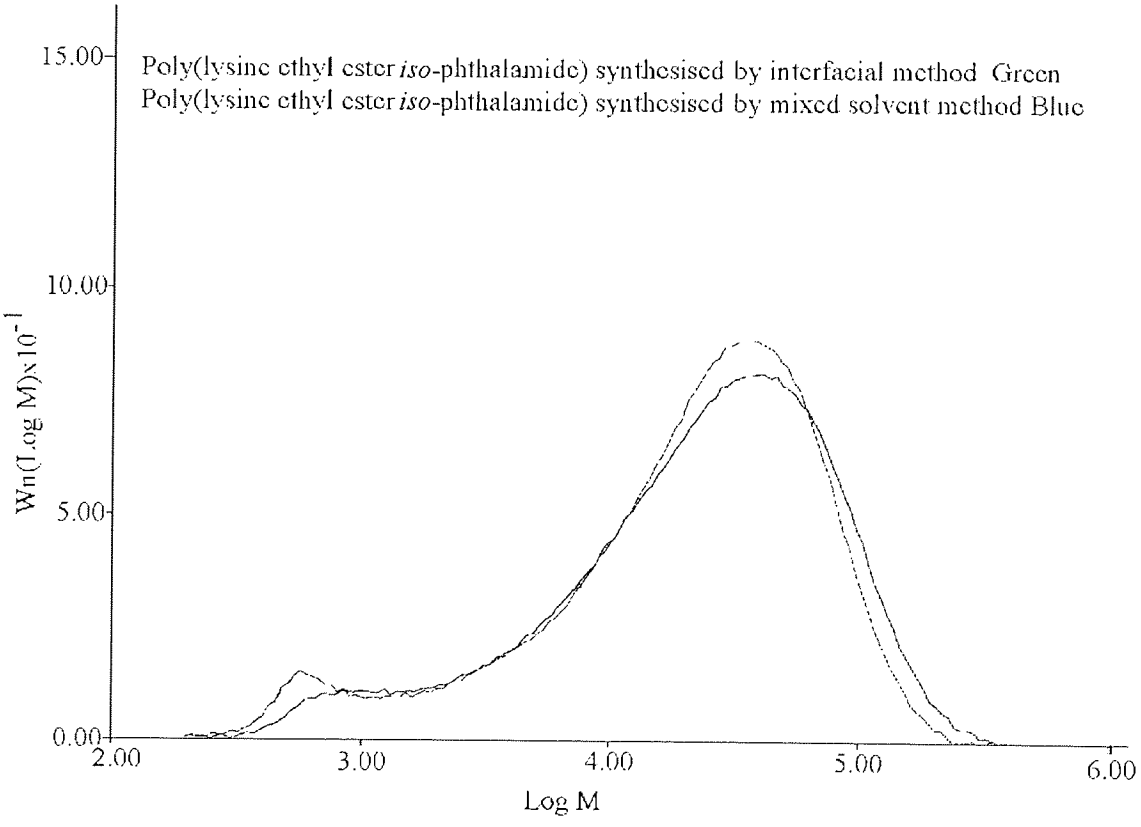
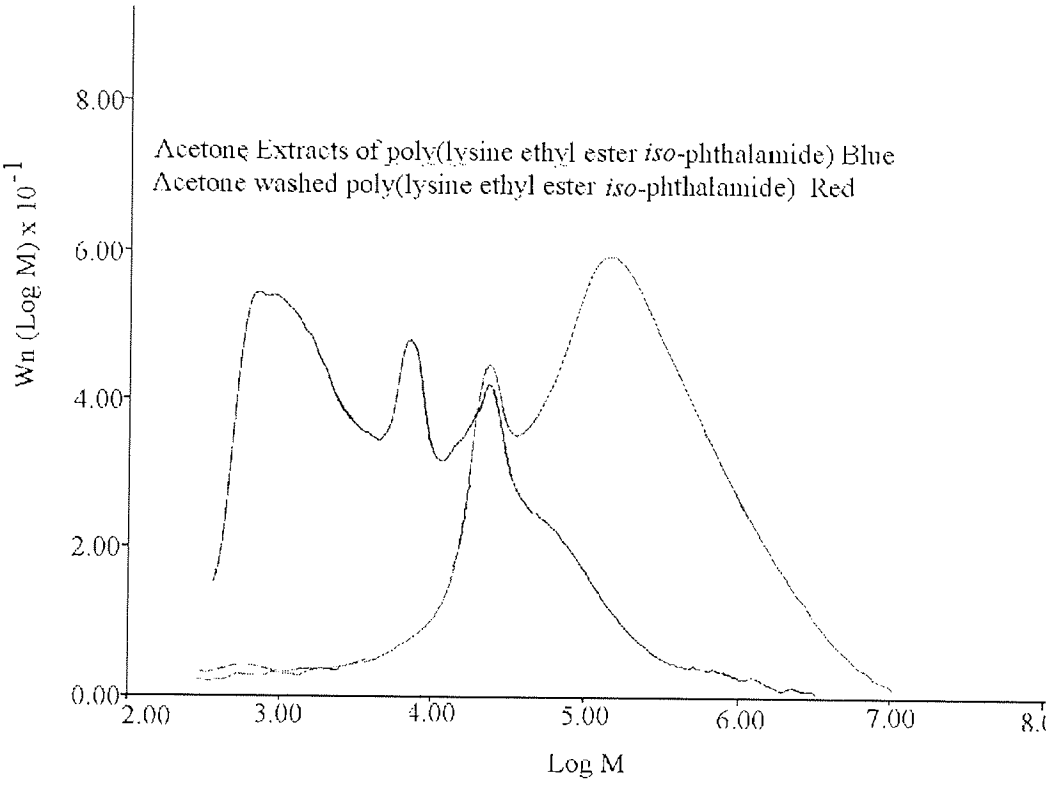


Fig. 5.6 Molecular weight distributions of interfacially synthesised poly (lysine ethyl ester iso-phthalamide) and cold acetone extracts.



5.5 VARIATION IN MOLECULAR WEIGHT DISTRIBUTION OF INTERFACIALLY SYNTHESISED POLY (LYSINE ETHYL ESTER *ISO*-PHTHALAMIDE) WITH REACTION CONDITIONS.

The molecular weight distributions of samples of interfacially synthesised poly (lysine ethyl ester *iso*-phthalamide) vary considerably with the conditions of synthesis (Fig. 5.7). Increasing the concentration of the two phases from 0.2M to 0.3M and the volume of each phase from 50mL to 300mL reduced the difference between the two peaks at high molecular weight but resulted in an increase in the relative intensity of the lower peak. Increasing the concentration of reagents would be expected to increase the rate of reaction and, therefore, the rate of precipitation. Gelation and a resultant reduction in stirring efficiency of the reaction medium would be more rapid although the higher concentration of reagents would increase diffusion through the solvent swollen precipitate.

This is supported by the fact that the lower molecular weight peak appears at higher molecular weight in the reaction with a higher monomer concentration. Soxolet extraction of the polymer synthesised using phase volumes of 300mL and monomer concentrations of 0.3M with chloroform was performed to remove low molecular weight oligomers. The molecular weight distributions of the crude sample, the extracted sample and the chloroform extracts were very similar in shape (Fig. 5.8) and the molecular weight averages suggested that the chloroform soluble extracts were higher in molecular weight than the insoluble fraction (Table 5.13). It is possible that all of the polymer would have eventually dissolved in chloroform. Acetone removes only low molecular weight oligomers and is, therefore, more suited as a solvent for polymer fractionation.

Fig. 5.7 Variation in molecular weight distributions of interfacially synthesised poly (lysine ethyl ester iso-phthalamide) with reaction conditions.

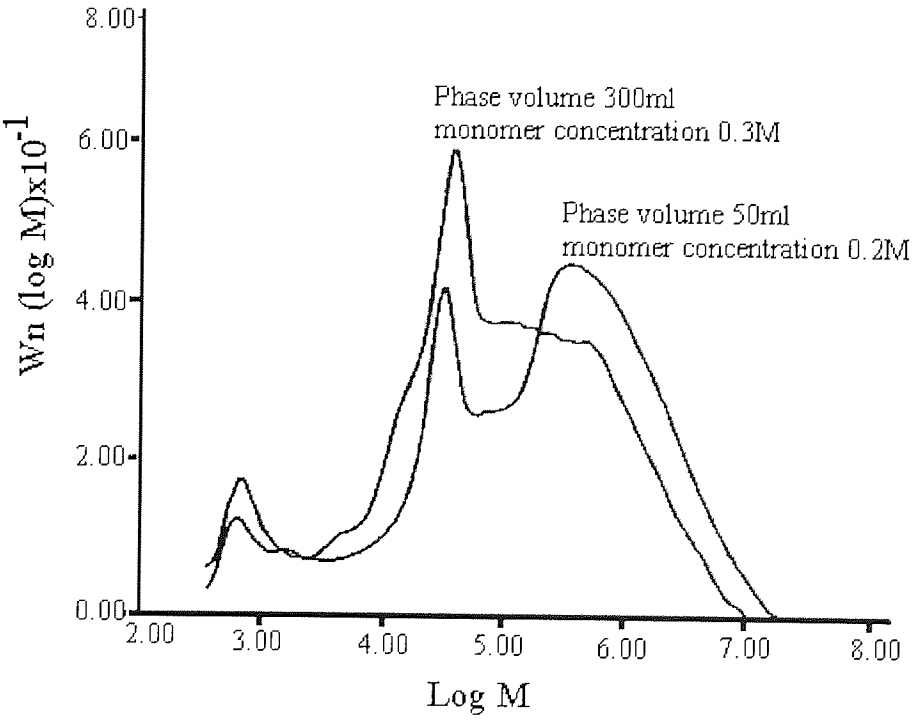
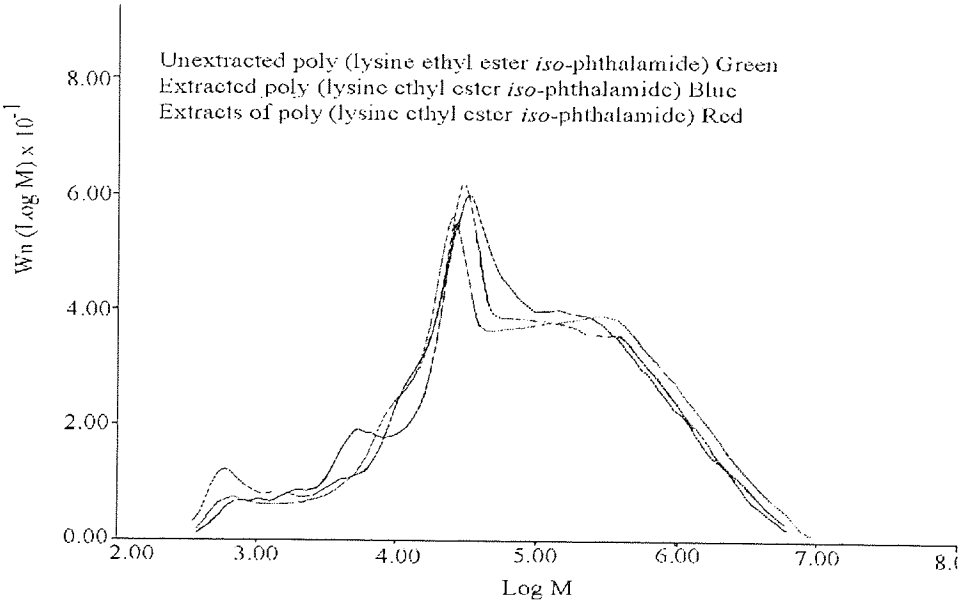


Table 5.13 Variation in molecular weight averages and polydispersisities of interfacially synthesised poly (lysine ethyl ester *iso*-phthalamide) with reaction conditions.

Samples Analysed 12.3.96 in DMF with ammonium acetate			
Sample Preparation	Mw 1st run 2nd run	Mn 1st run 2nd run	Mw/Mn 1st run 2nd run
ME 4.2.3.1	485000 549000	16300 19600	29.8 28.0
ME 4.2.3.2	52300 59820	2030 2150	25.8 27.8
ME 4.2.4.1	389000 394000	7660 8750	50.8 45.0
ME 4.2.4.2	355000 375000	10900 11100	32.6 33.4
ME 4.2.4.3	496000 494000	10600 10800	46.8 45.74

Fig. 5.8 Molecular weight distributions of poly (lysine ethyl ester iso-phthalamide) before and after extraction in chloroform.



5.6 MOLECULAR WEIGHT DISTRIBUTIONS OF INTERFACIAL POLYCONDENSATES BASED ON ALIPHATIC DIACYL CHLORIDES.

5.6.1 Molecular weight distributions of poly (lysine ethyl ester diethylmalonamide)

The reduction in molecular weight on switching from an interfacial to a single phase mixed solvent system when using hydrolytically sensitive diacyl chlorides was demonstrated by the polymerisation of the aliphatic diacyl chloride, diethylmalonyl chloride, with lysine ethyl ester. 2HCl (Table 5.14).

Table 5.14 Variation in molecular weight averages and polydispersisities of poly (lysine ethyl ester diethylmalonamide) with method of synthesis.

Samples Analysed 26.4.95 in DMF with ammonium acetate			
Sample Preparation	Mw 1st run 2nd run	Mn 1st run 2nd run	Mw/Mn 1st run 2nd run
ME 4.15.3	 8450 8680	 1260 1270	 6.7 6.8
ME 3.15.2	3150 3310	1190 1240	2.7 2.7

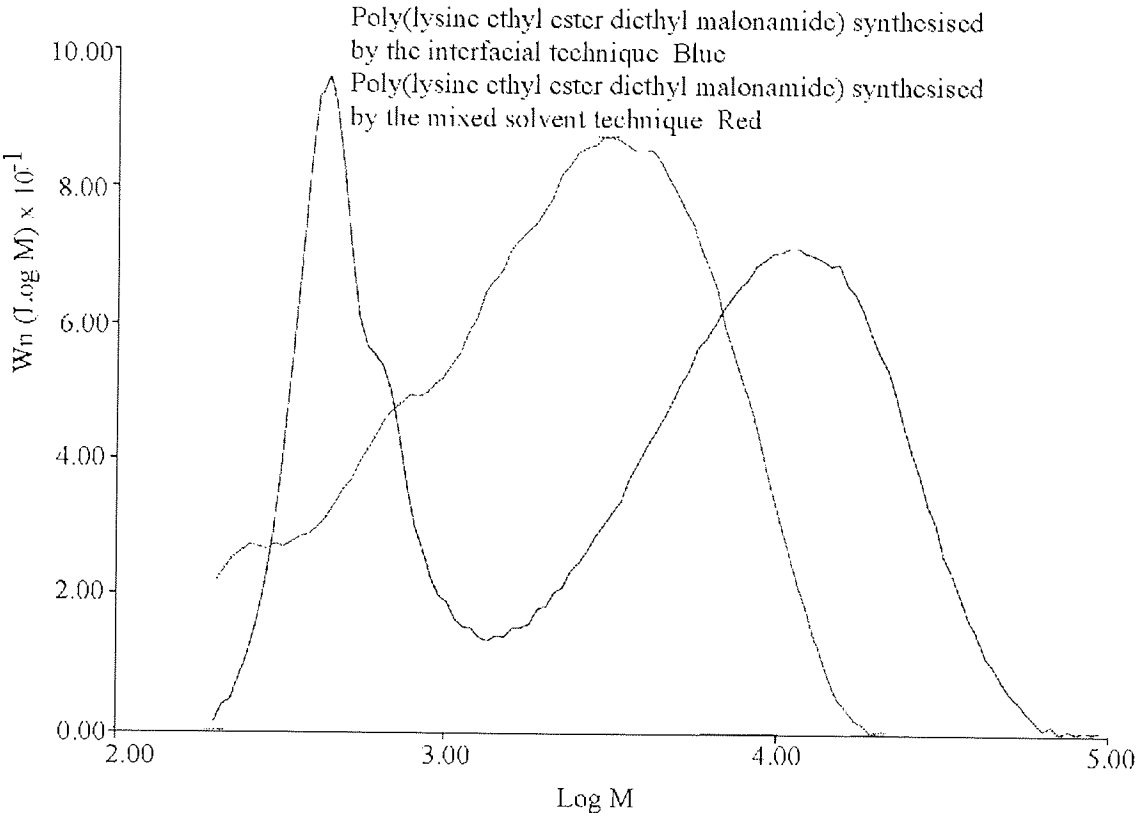
Examination of the raw chromatograms showed that interfacially prepared poly (lysine ethyl ester diethylmalonamide) had a bimodal molecular weight distribution whereas poly (lysine ethyl ester diethylmalonamide) prepared using a single phase mixed solvent system was unimodal (Fig. 5.9). A bimodal molecular weight distribution is usually indicative of one of the following:-

a) the sample is a blend

b) competing reaction mechanisms during polymerisation

c) degradation by a post-polymerisation process.

Fig. 5.9 Chromatogram of interfacially synthesised poly (lysine ethyl ester diethylmalonamide) and poly (lysine ethyl ester diethylmalonamide) synthesised by the miscible mixed solvent method.



There are several possible explanations for the presence of the peak at low molecular weight in the interfacially prepared poly (lysine ethyl ester diethylmalonamide). Rapid precipitation of the polymer resulted in the formation of an unstirrable mass and the formation of low molecular weight oligomers within such a precipitated mass is discussed in Ch.1. In the mixed solvent single phase synthesis rapid precipitation did not occur and the system remained stirrable throughout the duration of the reaction. In addition, in a single phase system, the reaction does not proceed at the interface of two immiscible liquids, precluding membrane formation. The resultant reduction in the rate of diffusion of the reagents to the site of polymerisation, that causes the formation of low polymer in some interfacial reactions, does not occur.

Alternatively the peak may be due to the presence of diethylmalonic acid produced by hydrolysis of unreacted diethylmalonyl chloride. This is possible because the polymer was precipitated in a highly solvent swollen state and unreacted monomer may have persisted in the bulk despite washing with both distilled water and excess organic solvent. However, this was also true of the single phase mixed solvent system and one may, therefore, expect that a similar peak at low molecular weight no such peak exists supporting the first explanation. Addition of water to the miscible solvent system after removal of the initial precipitate causes clouding. The mixed solvent system retains low molecular weight polymer in solution thus reducing the polydispersity of the final polymer.

The problems of long term reproducibility of G.P.C. are demonstrated by comparing the molecular weights of a sample of poly (lysine ethyl ester diethylmalonamide) obtained by GPC analysis on two different occasions (Table 5.15). Although the number average molecular weights compare favourably the weight average molecular weights show a large deviation resulting in a large difference between the polydispersisities. Similar deviations were seen between the molecular weight averages of poly (lysine ethyl ester phenylglutamide). The molecular weight averages and polydispersisities for poly (lysine ethyl ester phenylmalonamide) are included for comparison. The poly (lysine ethyl ester phenylmalonamide) sample was analysed when the other two samples were rerun. For the second analysis ammonium acetate was used in place of lithium bromide to reduce non steric interactions. Deviations in flow rate were higher than normally tolerated resulting in a lack of reproducibility in some samples and many of the samples analysed at this time showed a poor detector response. In such cases reproducibility between duplicate runs was expected to be poor because of noise on the system. Both increases and decreases in apparent molecular weight were seen between successive runs.

The results were further complicated by the presence of system peaks of varying intensity. The system peak closest to the polymer peak may be due to ammonium acetate, a view which is supported by a change in polarity of the peak (from positive to negative) with a change in solvent reservoir. Such a change may occur with small differences in the amount of ammonium acetate present in the solvent.

Table 5.15 Molecular weight averages and polydispersisities of poly (lysine ethyl ester diethylmalonamide), poly (lysine ethyl ester phenylglutamide) and poly (lysine ethyl ester phenylmalonamide) samples analysed at different times.

Sample	Method of Synthesis	Mw 1st run 2nd run	Mn 1st run 2nd run	Mw/Mn 1st run 2nd run
Poly (lysine ethyl ester diethylmalonamide) analysed 26.4.95	ME 4.15.3.1 (original run)	8450 8680	1260 1270	6.7 6.8
Poly (lysine ethyl ester diethylmalonamide) analysed 12.3.96	ME 4.15.3.1 (re run)	45500 20100	1240 1150	36.7 17.5
Poly (lysine ethyl ester phenylglutamide) analysed 13.12.94	ME 4.36.1 (original run)	6770 6650	2120 2140	3.2 3.1
Poly (lysine ethyl ester phenylglutamide) analysed 12.3.96	ME 4.36.1 (re run)	89400 137000	1430 1260	65.5 108.7
Poly (lysine ethyl ester phenylmalonamide)	ME 4.32.1 General Method 1	10500 7680	1390 1210	7.6 6.3

5.6.2 Effect of surfactants.

The molecular weight averages of interfacially prepared poly (lysine ethyl ester diethylmalonamide) were increased by the presence of the surfactant sodium lauryl sulphate (Table 5.16).

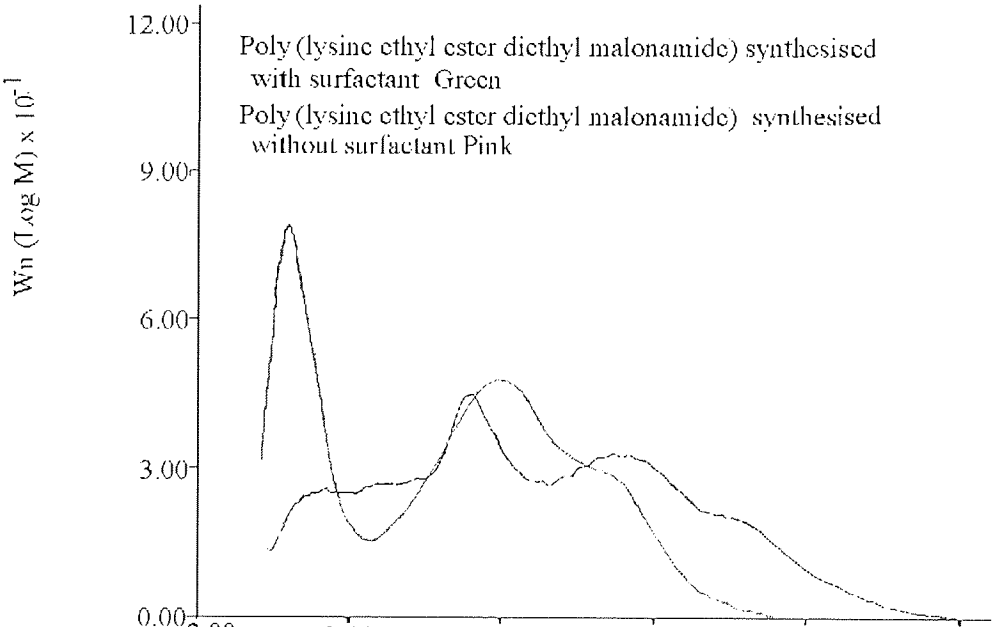
Table 5.16 Variation in molecular weight and polydispersity of poly (lysine ethyl ester diethylmalonamide) with addition of a surfactant.

Samples Analyzed12.3.96 Solvent: DMF with ammonium acetate			
Sample Preparation	Mw 1st run 2nd run	Mn 1st run 2nd run	Mw/Mn 1st run 2nd run
Interfacial Synthesis Organic Solvent CCl_4 [Acid Acceptor] 0.7M K_2CO_3 [Monomer] 50mL 0.2M ME 4.15.3.1	45500 20100	1240 1150	36.7 17.5
Interfacial Synthesis Organic Solvent CCl_4 [Acid Acceptor] 1.6M Na_2CO_3 [Monomer] 15mL 0.2M 1% sodium Laurate ME 4.22	284000 184000	2840 2840	100 64.8

The concentration of acid acceptor was higher in the case where surfactant was added but this would not account for the large increase in the molecular weight averages. This was attributed to increased stirrability throughout the reaction and to increased transfer of the diamine into the organic solvent.

The chromatogram of poly (lysine ethyl ester diethylmalonamide) prepared in the presence of a surfactant showed a clear shift towards higher molecular weight of the whole molecular weight distribution (Fig. 5.10). The molecular weight distribution was still bi-modal although the separation of the peaks is lower and the relative size of the low molecular weight peak is reduced when the surfactant was present. This would be expected if the stirring efficiency was improved since less low polymer would be formed. Increasing the concentration of surfactant further may eliminate the formation of low polymer giving a molecular weight distribution resembling that obtained for the mixed solvent single phase polycondensation which is essentially that of a solution polycondensation.

Fig. 5.10 Chromatograms of interfacially synthesised poly (lysine ethyl ester diethylmalonamide) with and without added surfactant.



5.7 MOLECULAR WEIGHT AVERAGES AND POLYDISPERSISITIES OF POLYAMIDES BASED ON HEXAMETHYLENE DIAMINE.

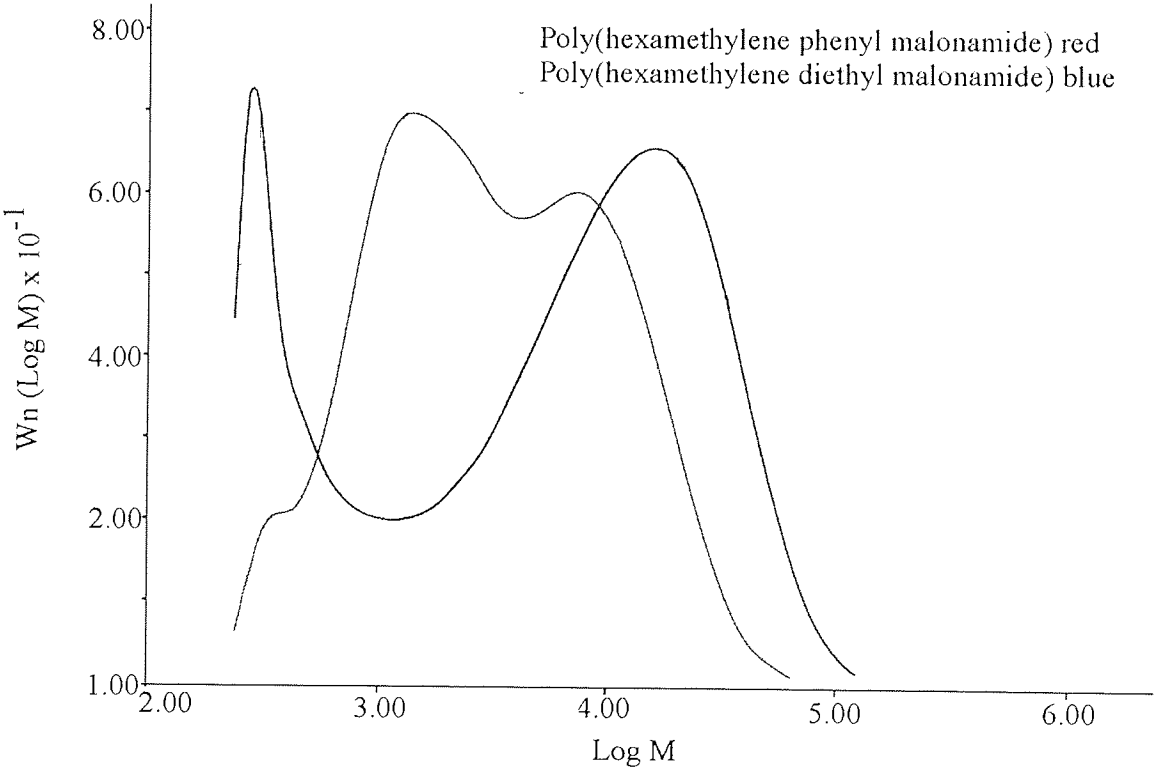
Direct comparison between the molecular weight averages of poly (hexamethylene diethylmalonamide) and poly (hexamethylene phenylmalonamide) should not be made because of the chemical dissimilarity between the diacyl segments of the repeat unit. It is possible to comment on the differences between the polydispersisities of the two samples (Table 5.17).

The polydispersity of poly (hexamethylene diethylmalonamide) was significantly higher than that of poly (hexamethylene phenylmalonamide) synthesised under similar conditions. The increased polydispersity of poly (hexamethylene diethylmalonamide) was attributed to the rapid formation of an unstirrable gelatinous precipitate. The formation of low polymer within such gels is discussed in Ch.1 and section 5.6.1 Examination of the raw chromatogram of poly (hexamethylene diethylmalonamide) shows a large peak at low molecular weight supporting this theory (Fig. 5.11). The chromatogram of poly (hexamethylene phenylmalonamide) showed a smaller peak at low molecular weight indicating that some formation of low polymer had occurred. In addition, the main peak in the chromatogram of poly (hexamethylene phenylmalonamide) is bimodal. A large peak appears at an apparent molecular weight of approximately 1500Da and may correspond to the formation of cyclic oligomers.

Table 5.17 Summary of molecular weight averages and polydispersisities of polyamides based on hexamethylene diamine.

Samples Analysed 12.1.94 in DMF with 0.01% LiBr				
Sample	Sample code	Mw 1st run 2nd run	Mn 1st run 2nd run	Mw/Mn 1st run 2nd run
Poly (hexamethylene diethylmalonamide)	ME 4.16	12700 12200	1300 1300	9.6 9.3
Poly (hexamethylene phenylmalonamide)	ME 4.34	6600 6800	1800 1700	3.7 3.9

Fig. 5.11 Chromatograms of interfacially synthesised poly (hexamethylene diethylmalonamide) and poly (hexamethylene phenylmalonamide).



5.8 MOLECULAR WEIGHT DISTRIBUTIONS OF INTERFACIAL POLYCONDENSATES BASED ON 1,3-BENZENE DI-SULPHONYL DICHLORIDE.

Polyamides of 1,3-benzene di-sulphonyl dichloride with lysine ethyl ester. 2HCl and lysine were prepared using the interfacial technique according to Sec. 4.4. and the mixed miscible solvent technique according to Sec. 3.7.

Poly (lysine 1,3-Benzene Di-Sulphonamide) and poly (lysine ethyl ester1,3-benzene di-sulphonamide) thus produced were found to be soluble in THF with gentle warming and so gel permeation chromatograms were obtained using an in house THF system at room temperature. The results are presented as polystyrene equivalents in Table 5.18

The molecular weights indicated by GPC support the findings of Beaumis *et. al.*[60] in that polysulphonamides can be successfully synthesised using lysine ethyl ester but not lysine as the diamine. This is attributed to the low solubility of lysine in the organic phase coupled with the low hydrolytic stability of 1,3-benzene di-sulphonyl dichloride. The reduced solubility of lysine impedes its availability for reaction in the organic phase. Therefore, in order for polymerisation to occur, the diacyl chloride must approach the aqueous phase. The low hydrolytic stability of 1,3-benzene di-sulphonyl dichloride means that hydrolysis prevents the formation of high molecular weight polymer. In this respect 1,3-benzene di-sulphonyl dichloride resembles the lower aliphatic acid chlorides which also fail to produce high polymer because of their rapid extraction and hydrolysis in the aqueous phase. The low hydrolytic stability of 1,3-benzene di-sulphonyl dichloride is demonstrated by the reduced molecular weight of poly (lysine ethyl ester 1,3-benzene di-sulphonamide) produced by the mixed solvent single phase method compared to the interfacial method.

Table 5.18 Summary of molecular weight averages and polydispersisities of polysulphonamides based on 1,3-benzene di-sulphonyl dichloride.

Samples analysed 26.4.96 Solvent; THF				
Sample	Method Of Synthesis	Mw	Mn	Mw/Mn
Poly (lysine ethyl ester 1,3-benzene di-sulphonamide)	2 Phase Initial Precipitate	38580	13125	2.9
Poly (lysine ethyl ester 1,3-benzene di-sulphonamide)	2 Phase Precipitate On Addition Of Hexane To Organic Phase	1051	707	1.5
Poly (lysine ethyl ester 1,3-benzene di-sulphonamide)	2 Phase Soxhlet Extracted With chloroform	73141	36766	2.0
Poly (lysine ethyl ester 1,3-benzene di-sulphonamide)	Mixed Solvent Single Phase	14100	3657	3.9
Poly (lysine 1,3-benzene di-sulphonamide)	2 Phase	519	347	1.5
Poly (lysine 1,3-benzene di-sulphonamide)	Mixed Solvent Single Phase	527	368	1.43

The fact that the mixed solvent single phase method fails to produce substantially higher molecular weight poly (lysine 1,3-benzene di-sulphonamide) than the interfacial method indicates that low availability of the diamine and hydrolytic sensitivity of the diacyl chloride are not the only two factors involved.

Zwitterion formation would lead to the formation of low oligomers because the charged NH_3^+ group would not be reactive towards the acyl chloride, however, under the conditions of the reaction the pH was sufficiently high to preclude Zwitterion formation. Both amine functions would be uncharged and available for reaction. Steric effects could play a part in reducing the activity of the α–amine although this would also be true for lysine ethyl ester. The basicity of the α–amine is reduced relative to the γ-amine because of the electron withdrawing effect of the α–carboxyl group. Sulphonamide formation would occur between the γ-amine of lysine and lysine ethyl ester at a similar rate whereas at the α–amine sulphonamide formation would be more rapid with lysine ethyl ester. 2HCl than with lysine. Under conditions where competing hydrolysis was a problem a fall in the reactivity of the diamine would lead to a fall in molecular weight.

6. CHAPTER 6

PHYSICAL PROPERTIES

6.1 SYNTHESIS OF MICROPARTICLES FROM POLY (LYSINE ETHYL ESTER *ISO*-PHTHALAMIDE).

Poly (lysine ethyl ester *iso*-phthalamide) microspheres were produced using a modified solvent extraction/evaporation technique. The technique was developed to produce microparticles from pre-synthesised poly (lysine ethyl ester *iso*-phthalamide) as opposed to their direct interfacial synthesis. Interfacial synthesis has been used in the direct synthesis of microcapsules from structurally related polymers[63-68], however, the optimum conditions for the production of high molecular weight polymer are not necessarily the same as those required for the successful production of microparticles. Indeed one of the main prerequisites in the formation of microcapsules is that the membrane formed around the droplets of the dispersed phase remain intact and this has been shown to result in the formation of low molecular weight polymer on the interior face of the membrane[8,12,17,18]. Additionally, the encapsulating membranes of microcapsules produced by the interfacial technique are generally too thin to act as an effective barrier to diffusion for extended drug delivery applications. The microcapsules produced are more suited as simple blood cell models[63,65]. It was reasoned that microspheres formed from the pre-formed polymer would be more easily reproduced and could be tailored to a particular application by modification of the polymer prior to and independently of the microspherulisation process. For example, Kondo *et. al.*[67] demonstrated that increased stirring speeds resulted in an increased equilibrium partition coefficient of the diamine in the organic phase. An increase in the speed of stirring would also result in the formation of a finer dispersion and thus a greater amount of interfacial area. All of these factors would lead to an increase in the rate of polymerisation coupled with a decrease in the size of the microspheres. The thickness of the membrane relative to the encapsulated solvent reservoir would also vary. Recently, Zhang *et. al.*[159] have investigated the effects of variables such as speed, concentration and nature of emulsifying agent, phase volume ratio and emulsification time of the oil and aqueous phase prior to addition of the amine moiety on the size distribution of microspheres based on phthaloyl dichlorides and diethylenetriamine.

Narrower distributions in size were obtained with increasing concentrations of surfactant and longer pre-polymerisation emulsification times. Decreases in average size distribution were also noted. Modification of the experimental conditions would allow the synthesis of both microspheres and microcapsules, whereas the interfacial process is only applicable to the production of micro capsules[159]. Kondo *et. al.*[67] point out that it should be possible to produce solid monolithic microparticles under conditions where the concentration of the diacid chloride in the organic phase is relatively high since the locus of polymerisation would then be within the bulk of the organic solvent rather than at the interface. The range of suitable candidates for inclusion in the microparticles would also be extended by using pre-formed polymer since potential reaction with the diacyl chloride precludes the encapsulation of species containing active protons by the interfacial process.

Microspheres are routinely made by solvent evaporation or solvent extraction processes as described in section 2.3.1 Both these methods produce microspheres by the removal of solvent from an organic solution of the polymer dispersed within a continuous immiscible phase (typically mineral oil). Solvents with relatively low boiling points, such as dichloromethane (bp 41°C), are generally employed so that solvent evaporation can take place without excessive heating of the dispersion which may lead to increased tackiness and agglomeration of the microspheres or degradation of thermally sensitive pharmacons. Reduced pressure is often employed to lower the temperature required for hardening of the microspheres when solvents with higher boiling points such as acetonitrile (bp 81-82°C) are used. Poly (lysine ethyl ester *iso*-phthalamide) was found to be soluble only in DMF (bp 153°C) and DMSO (bp 189°C). This effectively rules out solvent evaporation even under reduced pressure because of the high temperatures that would be required. Solvent extraction requires a non solvent for the polymer that is miscible with both the continuous phase and the dispersed phase. Complete removal of the polymer solvent is required to harden the microspheres and prevent coagulation during the isolation process which generally involves filtration and/or centrifugation.

Distilled water was found to be a suitable non solvent for poly (lysine ethyl ester *iso*-phthalamide) and caused rapid precipitation from solution in DMF. Solvent extraction results in the formation of microspheres by removal of the solvent from the dispersed droplets of polymer solution. The solvent is extracted into the continuous phase, leaving a dispersion of solid particles in a mixture of the original solvents and the non solvent. Distilled water is obviously immiscible with mineral oil and so a conventional solvent extraction technique cannot be applied.

Poly (lysine ethyl ester *iso*-phthalamide) was dissolved in a DMF/chloroform mixture close to the cloud point. This solution was mixed with distilled water and a surfactant which caused extraction of the DMF into the continuous aqueous phase and precipitation of the polymer within the dispersed droplets of chloroform. From the interfacial synthesis of poly (lysine ethyl ester *iso*-phthalamide) it was noted that chloroform (and other organic solvents used) swelled the precipitated polymer. The microspheres were thus hardened by heating the dispersion to 40°C for 2 hours to evaporate the chloroform.

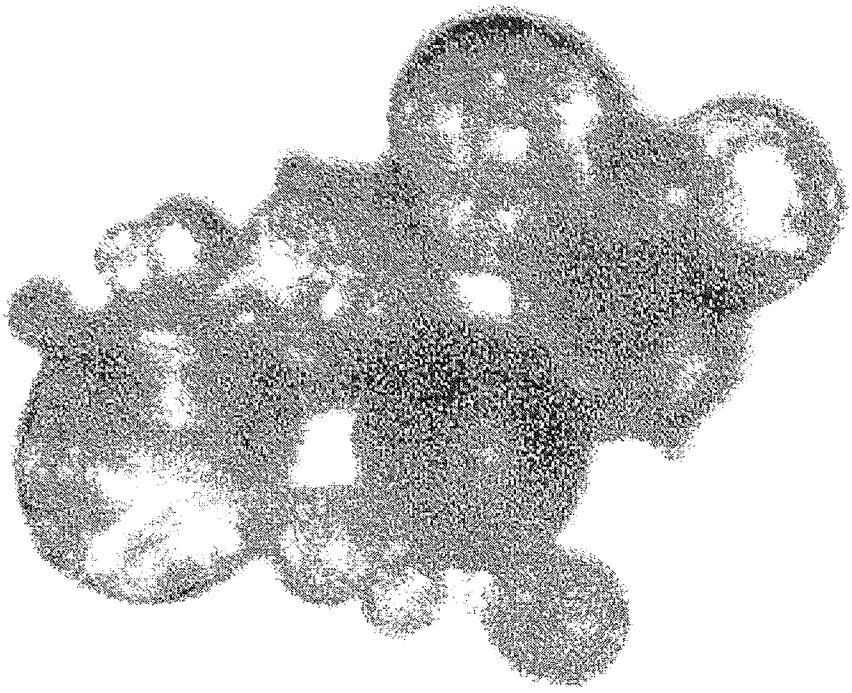
The technique is similar to that employed by Coombes *et. al.*[109] described in Sec. 2.3.2 for the synthesis of polylactide-co-glycolide microspheres using PEO-PPO stabilisers. A mixed solvent system consisting of 1:1 acetone:dichloromethane was used to dissolve the polylactide-co-glycolide which was then emulsified with an aqueous solution of the stabiliser. Rapid extraction of the acetone resulted in the precipitation of the polymer at the interface of the dispersed and continuous phases. The extraction of the acetone into the aqueous phase also caused shrinkage of the dispersed droplets with consequent entrapment of the hydrophobic portion of the stabiliser molecules in the surface of the microspheres. The microspheres were subsequently hardened by evaporation of the dichloromethane. The method of Coombes *et. al.*[109] differs slightly from the method selected for the production of poly (lysine ethyl ester *iso*-phthalamide) microspheres in that the acetone would also be lost by evaporation whereas dimethylsulphoxide would remain in the aqueous phase

A related hybrid technique is described by Gardner[160] in a patent filed in 1987 for the production of microcapsules from homo- and copolymers of glycolic and lactic acids. The polymer is dissolved in a solvent/non solvent mixture and emulsified with an aqueous solution of the material to be encapsulated. Application of heat causes the solvent/non solvent mixture to evaporate. The solvent was chosen to have a higher vapour pressure than the non solvent so that as the mixture evaporates the proportion of non solvent increases resulting in precipitation of the polymer around the dispersed aqueous droplets. After encapsulation is complete a miscible non solvent is added to the system to extract the residual solvent from the polymer membrane

6.1.1 Synthesis of microspheres using polyvinyl alcohol as the emulsifying agent.

50mL of dichloromethane was added to 10mL of 10% poly (lysine ethyl ester *iso*-phthalamide) in DMF causing the solution to become opaque. DMF was then added until the solution just became transparent (approximately 2mL). The solution of polymer in DMF/dichloromethane was stirred in a 250mL round bottomed flask at 2000 r.p.m. with an overhead stirrer and 100mL of 0.5% aqueous polyvinyl alcohol solution was added rapidly. Precipitation of the polymer occurred rapidly and the dispersion was heated with continued stirring for two hours to harden the microspheres. The dispersion was then added to 250mL of distilled water and centrifuged at 1000 r.p.m. The liquid was decanted off and a white solid was redispersed in 250mL distilled water and recovered by filtration. The white solid was dried in a vacuum oven at 55°C overnight to give a white powder which was examined by optical microscope. The powder was seen to consist of roughly spherical particles ranging in size from to 18-100μm. The majority of the particles were coagulated into clumps of 10 or more (Fig 6.1).

Fig. 6.1 Poly (lysine ethyl ester iso-phthalamide) microspheres produced using poly (vinyl alcohol) as emulsifier.



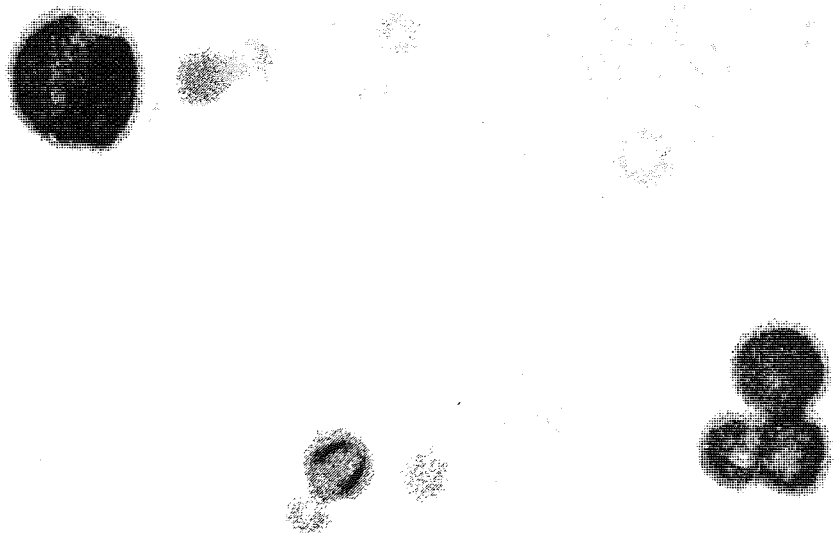
It was apparent that the degree of sphericity was low. This was attributed to the high viscosity of the organic phase and to rapid removal of the DMF from the organic solvent system. The coagulation of the particles may have occurred because of the high temperature used to remove the dichloromethane.

The experiment was repeated with 2% polyvinyl alcohol as emulsifier. The higher concentration of emulsifier was used to reduce coagulation of the microspheres. The total volume of the solution of poly (lysine ethyl ester *iso*-phthalamide) was increased to 75mL. 13mL of DMF were required to maintain a transparent solution. The microspheres were isolated as in the previous experiment. Examination of the dried powder showed clumps of roughly spherical particles with similar diameters as in the previous experiment.

6.1.2 Synthesis of microspheres using sodium oleate as the emulsifying agent.

50mL of dichloromethane was added to 10mL of 5% poly (lysine ethyl ester *iso*-phthalamide) in DMF. The solution of polymer in DMF/dichloromethane was stirred in a 250mL round bottomed flask at 2000 r.p.m. with an overhead stirrer and 100mL of 2% sodium oleate solution was added rapidly. The emulsion was heated to 40°C for two hours after which time stirring was stopped. A white powder settled at the bottom of the round bottomed flask leaving a straw coloured suspension. The suspension was filtered under gravity and the white powder thus isolated was redispersed in 250mL distilled water. Examination of the powder by optical microscope (Fig. 6.2) showed mainly spherical particles with a narrower size distribution than when poly (vinyl alcohol) was used as the surfactant (particle diameters 25-50μm). The powder was not isolated by centrifuge since this was seen as a contributing factor to the coagulation of the microspheres. Reducing the concentration of the organic phase gave a solution of lower viscosity which may have contributed to the improved sphericity of the microspheres although changing the emulsifier may also have had an effect. It was reasoned that the use of a charged surfactant would reduce coagulation of the microspheres because of ionic repulsion between the charged groups of surfactant molecules absorbed to the surfaces of the spheres.

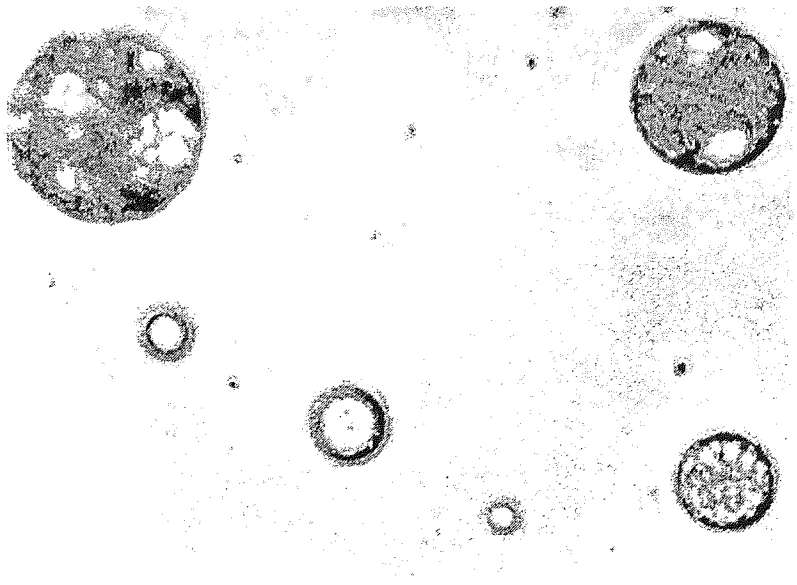
Fig. 6.2 Poly (lysine ethyl ester iso-phthalamide) microspheres produced using sodium oleate as emulsifier.



6.1.3 Synthesis of microspheres using polyvinylalcohol and methylcellulose as the emulsifying agents.

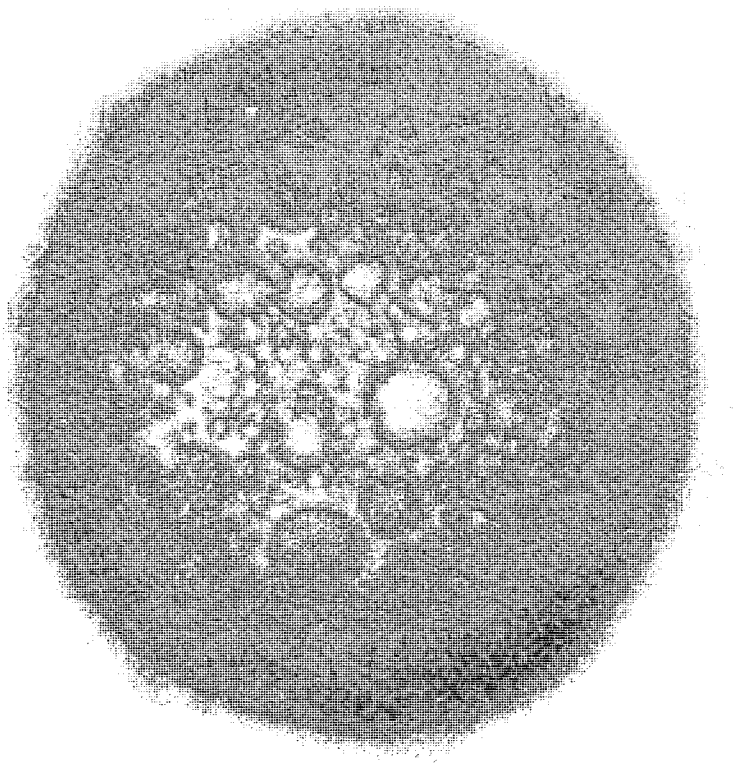
50mL of dichloromethane was added to 10mL of 10% poly (lysine ethyl ester *iso*-phthalamide) in DMF causing the solution to become opaque. DMF was then added until the solution just became transparent (approximately 2mL). The solution of polymer in DMF/dichloromethane was stirred in a 250mL round bottomed flask at 2000 r.p.m. with an overhead stirrer and 100mL of 2% aqueous polyvinyl alcohol/0.5% methyl cellulose solution. The emulsion was heated to 40°C for two hours after which time stirring was stopped. The resulting suspension was added to 250mL of distilled water and filtered under gravity. Filtration proceeded extremely slowly and the suspension was diluted with an additional 500mL of distilled water. Filtration then proceeded at a reasonable speed. The white powder thus obtained was redispersed in 250mL of distilled water and refiltered. After drying overnight in a vacuum oven a free flowing white powder was obtained. Examination of the powder by optical microscope (Fig 6.3) showed that the microparticles had a high degree of sphericity with a narrow size distribution (particle diameter 30-60μm) and showed little coagulation compared to the microcapsules synthesised using poly (vinyl alcohol) as stabiliser.

Fig. 6.3 Poly (lysine ethyl ester iso-phthalamide) microspheres produced using poly (vinyl alcohol) and methyl cellulose as emulsifier.



Closer examination of the microspheres under higher magnification reveals a highly honeycombed structure (Fig 6.4). Spenlehauer *et. al.*[162] observed a similar structure in microcapsules of poly (lactic acid) produced by the solvent evaporation method when small quantities of cyclohexane where included in the original solution of the polymer in dichloromethane. The less volatile cyclohexane is a poor solvent for the polymer and is entrapped within the solid microcapsules on evaporation of the dichloromethane. The porous structure is generated on removal of the residual cyclohexane. In a similar way extraction of the DMSO into the continuous aqueous phase leaves chloroform, a poor solvent for poly (lysine ethyl ester *iso*-phthalamide), trapped within the polymer matrix. Removal of the chloroform in the final solvent evaporation step leaves a honeycombed polymer with a porous surface. Control over the amount of chloroform and rate of chloroform evaporation may provide a mechanism for producing microparticles with tailored architectures.

Fig. 6.4 Honeycombed structure within poly (lysine ethyl ester iso-phthalamide) microsphere.



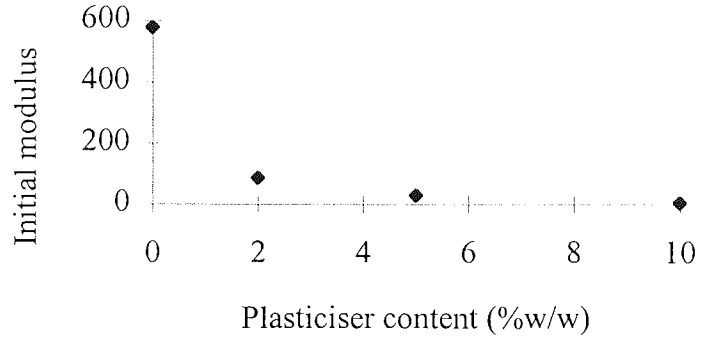
6.2 PHYSIOCHEMICAL PROPERTIES OF POLY (LYSINE ETHYL ESTER *ISO*-PHTHALAMIDE) MEMBRANES PLASTICISED WITH POLYCAPROLACTONE.

The film forming ability of a polymer and the mechanical properties of the film are important considerations for polymers intended for pharmaceutical coatings. The polymer must form an intact coherent film with is sufficiently robust to survive storage. Thus membranes for tensile testing were prepared from poly (lysine ethyl ester *iso*-phthalamide) synthesised using the mixed miscible solvent technique according to Sec. 3.2. Membranes were prepared by dissolving 4g of poly (lysine ethyl ester *iso*-phthalamide) in the minimum amount of warm DMF (approximately 20mL at 50°C). This was mixed with the appropriate amount of polycaprolactone in warm chloroform (approximately 2mL at 50°C). The solution was filtered through a preheated Buchner funnel and cast onto polycarbonate sheets within a rectangular a mould. The solutions were left to evaporate for 3 days in a fume cupboard at room temperature, then dried overnight in a vacuum oven at 55°C. Transparent films were prepared using 0%, 2%, 5% and 10% w/w polycaprolactone. Phase separation occurred with plasticiser contents of 25% w/w or above, leaving white granules of polycaprolactone dispersed throughout the film. Attempts to accelerate film formation by heating the cast solutions resulted in bubble formation within the film due to evaporation of the chloroform. The films produced showed a mottled effect indicating phase separation of the plasticiser and polymer. Films of poly (lysine *iso*-phthalamide) were produced using the same method. Transparent films were produced with plasticiser contents of 0%, 2% and 5% w/w but in all cases the films proved to be to brittle to allow separation from the polycarbonate sheets on which they were cast.

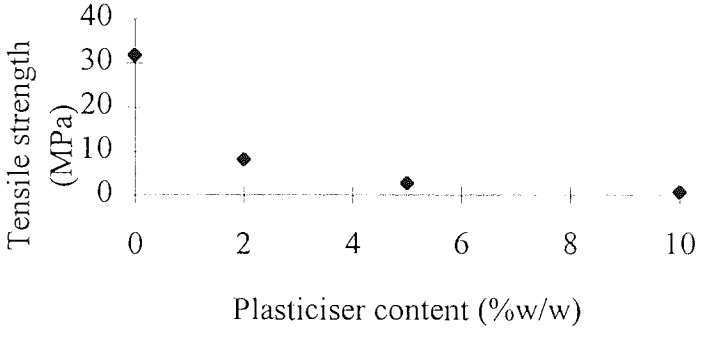
Tensile testing was performed on a Hounsfield Hti tensiometer at an extension speed of 20mm/min using dumbbell shaped samples. The central test section was 8mm long and 3.3mm wide. The sample was positioned so that the test section just protruded from the jaws of the tensiometer. The results obtained for the variation in initial modulus, tensile strength and elongation at break with plasticiser content represent the averages of four tests giving similar stress/strain curves and are displayed in graphs 6.1-6.3.

Some samples showed considerable variation in tensile properties with thickness and possible reasons for this are discussed in Sec. 6.2. The data plotted in graphs 6.1-6.3 is summarised in Appendix 3, Table C1.

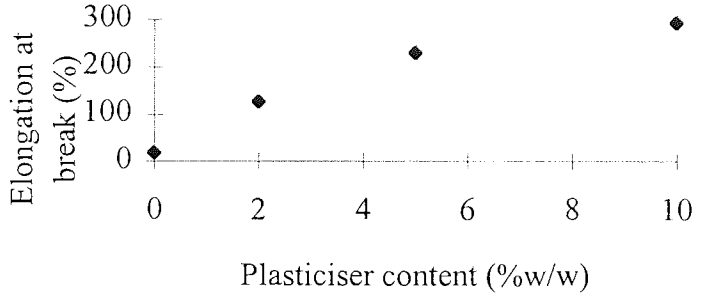
Graph 6.1 Variation of initial modulus of poly (lysine ethyl ester *iso*-phthalamide with plasticiser content.



Graph 6.2 Variation in tensile strength of poly (lysine ethyl ester *iso*-phthalamide) with plasticiser content.

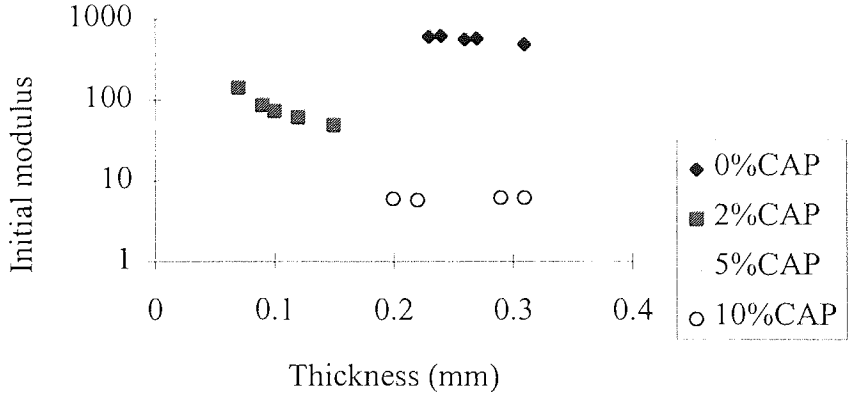


Graph 6.3 Variation of elongation at break of poly (lysine ethyl ester *iso*-phthalamide) with plasticiser content.

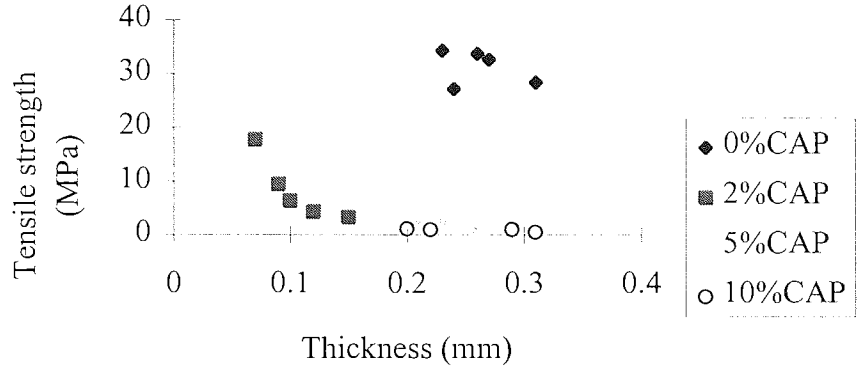


2 % w/w polycaprolactone had a large effect on the initial modulus and tensile strength of poly (lysine ethyl ester *iso*-phthalamide) compared to unplasticised poly (lysine ethyl ester *iso*-phthalamide) whilst increasing the plasticiser content further had only a marginal effect. Increasing the plasticiser content caused a progressive increase in the elongation at break. The variation in tensile properties with sample thickness are shown in graphs 6.4-6.6. The trends in graphs 6.1 and 6.2 can clearly be seen in graphs 6.4 and 6.5 despite apparent changes in tensile properties with sample thickness. The increase in elongation at break at higher plasticiser contents is less obvious in Graph 6.6 than in Graph 6.3 with a range of values for all plasticised samples occurring in the low to mid hundreds. There appeared to be a strong dependence between tensile properties and sample thickness for the samples containing 2 and 5% plasticiser whilst virtually no deviations were seen in the sample containing 10% plasticiser. Samples of increasing thickness had a lower initial modulus and lower tensile strength indicating higher plasticiser content. This was supported by the increase in elongation at break with sample thickness seen in the membrane containing 5% polycaprolactone but the sample containing 2% polycaprolactone showed a decrease in elongation at break with increasing sample thickness. The membrane containing 2% polycaprolactone was roughly half the average thickness of the other membranes because of leakage that occurred during the solvent evaporation process.

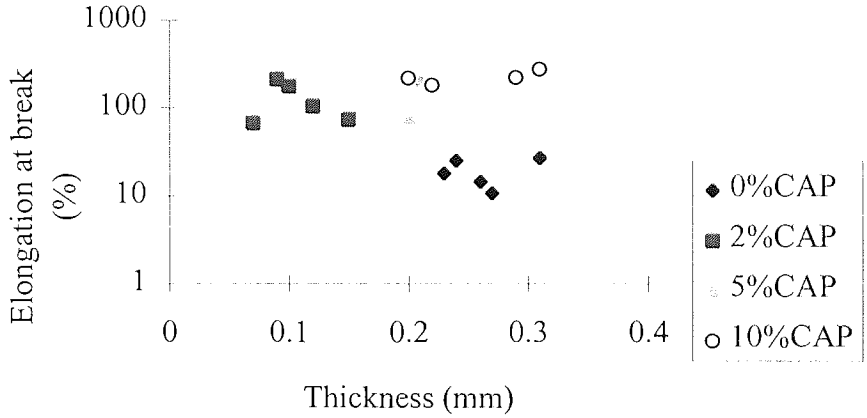
Graph 6.4 Variation in initial modulus of poly (lysine ethyl ester *iso*-phthalamide) with sample thickness.



Graph 6.5 Variation in tensile strength of poly (lysine ethyl ester *iso*-phthalamide) with sample thickness.



Graph 6.6 Variation in elongation at break of poly (lysine ethyl ester *iso*-phthalamide) with sample thickness.



The deviations in tensile properties with membrane thickness may have resulted from diffusion of the plasticiser within the membrane as the plasticiser solvent, chloroform, evaporated. The rate of evaporation of the chloroform would be lowest in the thicker regions of the membranes and so in view of the relatively low compatibility between the plasticiser and polymer, the plasticiser may diffuse to these chloroform rich regions. The relatively high boiling point of DMSO would maintain the polymer matrix in a solvent swollen state as the more volatile chloroform evaporated, facilitating diffusion of the plasticiser.

Diffusion of plasticizer into the thicker regions of the membranes would result in regionally high concentrations of plasticiser which may result in phase separation. It was noted that bubbles formed in samples that were not adequately air dried prior to drying in a vacuum oven. The bubbles resulted from the evaporation of the chloroform as was demonstrated by the lack of bubble formation in unplasticised films cast from DMSO only which were dried immediately in a vacuum oven without bubble formation. The films were all dried to constant weight in a vacuum oven to avoid plasticisation by residual solvent. Formation of bubbles within the membranes would be expected to have a detrimental effect on the tensile properties.

6.3 POTENTIOMETRIC AND SOLUBILITY RESULTS.

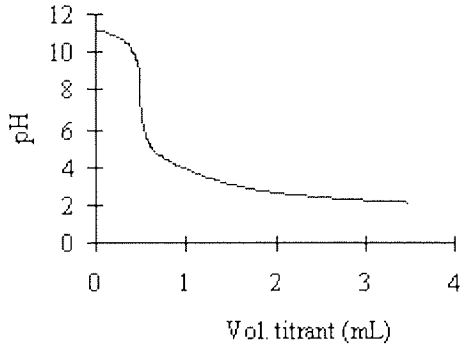
Potentiometric titrations were carried out on a Tacussel autotitrator equipped with a Camomel combined electrode and a 10 mL automatic burette. The neutralised polyamides (produced according to the methods outlined in Ch. 3 and Ch. 4) were dissolved with the addition of a slight excess of 0.1001M sodium hydroxide. The required amount of sodium chloride was added and the solution made up to 50mL in a volumetric flask. 25 mL of a 0.1% solution of the polyamide in 0.1M sodium chloride was titrated with 0.1019 M hydrochloric acid. The volume of titrant added varied between 5μL and 25μL such that a maximum number of points was obtained in the region of the inflexions and readings were taken when a critical stability of ± 0.05 pH units was reached or 30 seconds after addition of the titrant if this arbitrary stability was not achieved. The pH at the onset of precipitation was taken as the pH at which the titrated solution of polymer just became cloudy. The results are presented in Table 6.1. An additional sample of poly (lysine *iso*-phthalamide) was dialysed against distilled water using a dialysis sack with a molecular weight cut off of 12,000 to remove low molecular weight material.

Table 6.1 pH at onset of precipitation of polyamides containing pendent carboxylic acids.

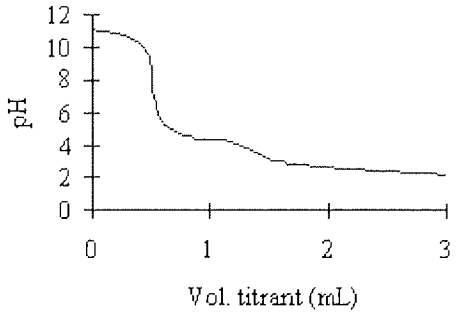
Sample	Sample Code	pH at onset of precipitation
Poly (ornithine *iso*-phthalamide)	ME 3.10	4.13
Poly (lysine *iso*-phthalamide)	ME 3.9	4.39
Dialysed Poly (lysine *iso*-phthalamide)	ME 3.9	4.40
Poly (lysine co lysine ethyl ester *iso*-phthalamide)	ME 3.12.2	4.47
Poly (lysine co hexamethylene *iso*-phthalamide) Aqueous soluble fraction	ME 4.4.2	4.75
Poly (lysine dodecanamide) (CCl_4 as organic solvent)	ME 4.27	5.00
Poly (ornithine dodecaneamide) (hexane as organic solvent) (chloroform as organic solvent)	 ME 4.30 ME 4.29	 4.85 5.20
Hydrolysed Poly (lysine ethyl ester phenylmalonamide)	ME 4.32.2	4.38
Hydrolysed Poly (lysine ethyl ester phenylglutamide)	ME 4.36.2	4.70
Hydrolysed Poly (lysine ethyl ester diethylmalonamide)	ME 4.15.3.2	4.00

From the potentiometric titrations of the polyamides synthesised in this thesis it was apparent that the polymers could be divided into two groups. One group displayed behaviour typical of a monobasic polyelectrolyte in that a single equivalence point was found whilst the second group displayed two equivalence points. Poly (lysine diethylmalonamide) was in the first group and a single equivalence point was seen in the plot of pH against volume of added titrant (Graph 6.7) at a pH of 9.1. A plot of the variation in pH with degree of ionisation showed a progressive increase in the degree of ionisation with increasing pH (Graph 6.8) and the apparent pKa increased linearly with increasing degree of ionisation (Graph 6.9). Poly (*iso*-phthalamides) based on lysine and ornithine fall into the second group and exhibit two equivalence points (Graph 6.10). In addition to the potentiometric titration, plots of the variation of pH and apparent pKa with degree of ionisation (graphs 6.11 and 6.12) also show abnormalities at low degrees of ionisation.

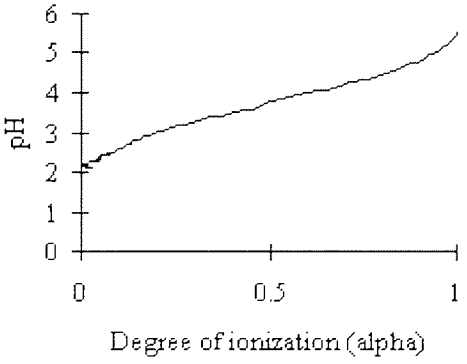
Graph 6.7 Variation in pH with volume of added titrant for poly (lysine diethylmalonamide).



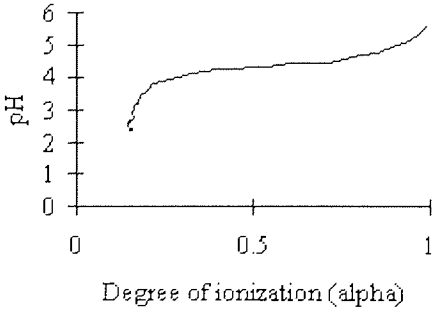
Graph 6.10 Variation in pH with volume of added titrant of dialyzed poly (lysine *iso*-phthalamide) (Mw > 12,000).



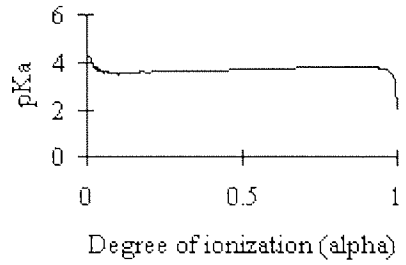
Graph 6.8 Variation in pH with degree of ionization of poly (lysine diethylmalonamide).



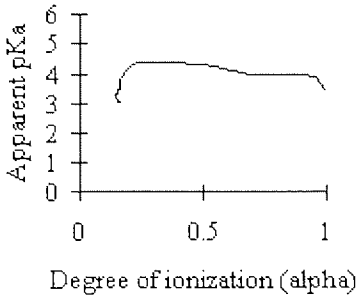
Graph 6.11 Variation in pH with degree of ionization of dialyzed poly (lysine *iso*-phthalamide) (Mw > 12,000).



Graph 6.9 Variation in apparent pKa of poly (diethyl malonamide) with degree of ionization (alpha).

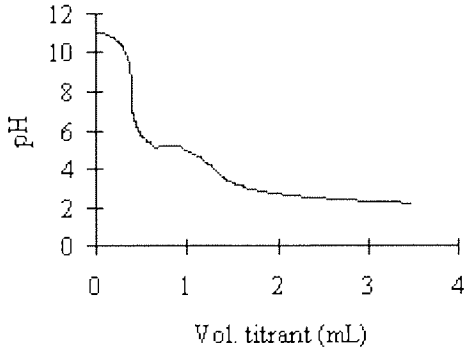


Graph 6.12 Variation in apparent pKa of dialyzed poly (lysine *iso*-phthalamide) with degree of ionization (Mw > 12,000).

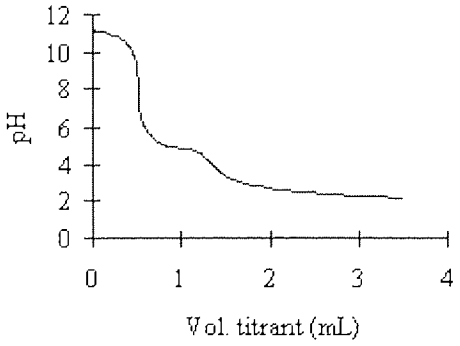


The potentiometric titrations of poly (lysine dodecanamide) (graphs 6.13-6.16) and poly (ornithine dodecanamide) (graphs 6.17-6.20) followed the trends seen in the *iso*-phthalamide polyelectrolytes also displaying two distinct points of inflexion.

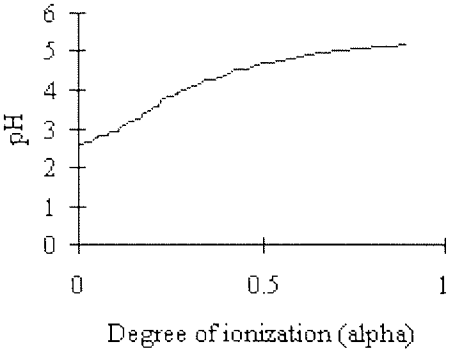
Graph 6.13 Variation in pH with volume of added titrant of poly (lysine dodecanamide).



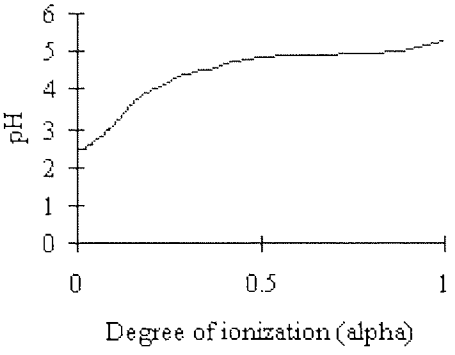
Graph 6.16 Variation in pH with volume of added titrant of poly (ornithine dodecanamide).



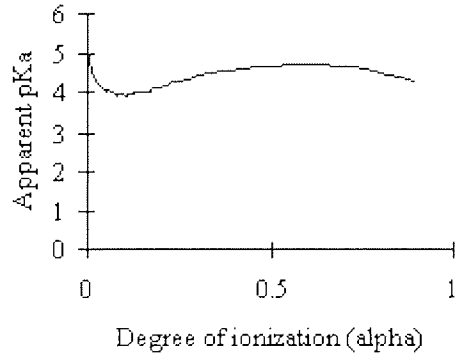
Graph 6.14 Variation in pH with degree of ionization for poly (lysine dodecanamide).



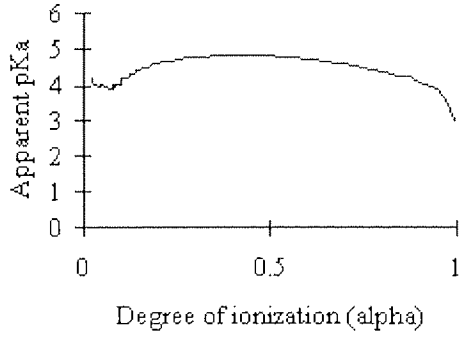
Graph 6.17 Variation in pH with degree of ionization of poly (ornithine dodecanamide).



Graph 6.15 Variation in apparent pKa with degree of ionization of poly (lysine dodecanamide).



Graph 6.18 Variation in apparent pKa of poly (ornithine dodecanamide) with degree of ionization.



Before discussing the results of the potentiometric titrations it would be useful to briefly consider the physiochemical properties of aqueous solutions of polyelectrolytes. Unlike unimeric mono basic acids which have a single pKa value, polyacids have a range of apparent pKa values depending on the degree of ionisation of the polymer. Thus, the apparent pKa of a normal polyacid increases with the degree of ionisation of the polymer[164,165]. The increase in pKa with degree of ionisation results from the increasing electrostatic repulsion experienced by the carboxylate ions along the polymer backbone as successive carboxylic acid groups are ionised. Polyacrylic acid is a typical polyacid showing such behaviour and the titration curves fit well with predicted curves for flexible polyelectrolytes that exhibit no secondary structure[166]. Formation of some secondary structure causes deviations from the theoretical curve[166,167].

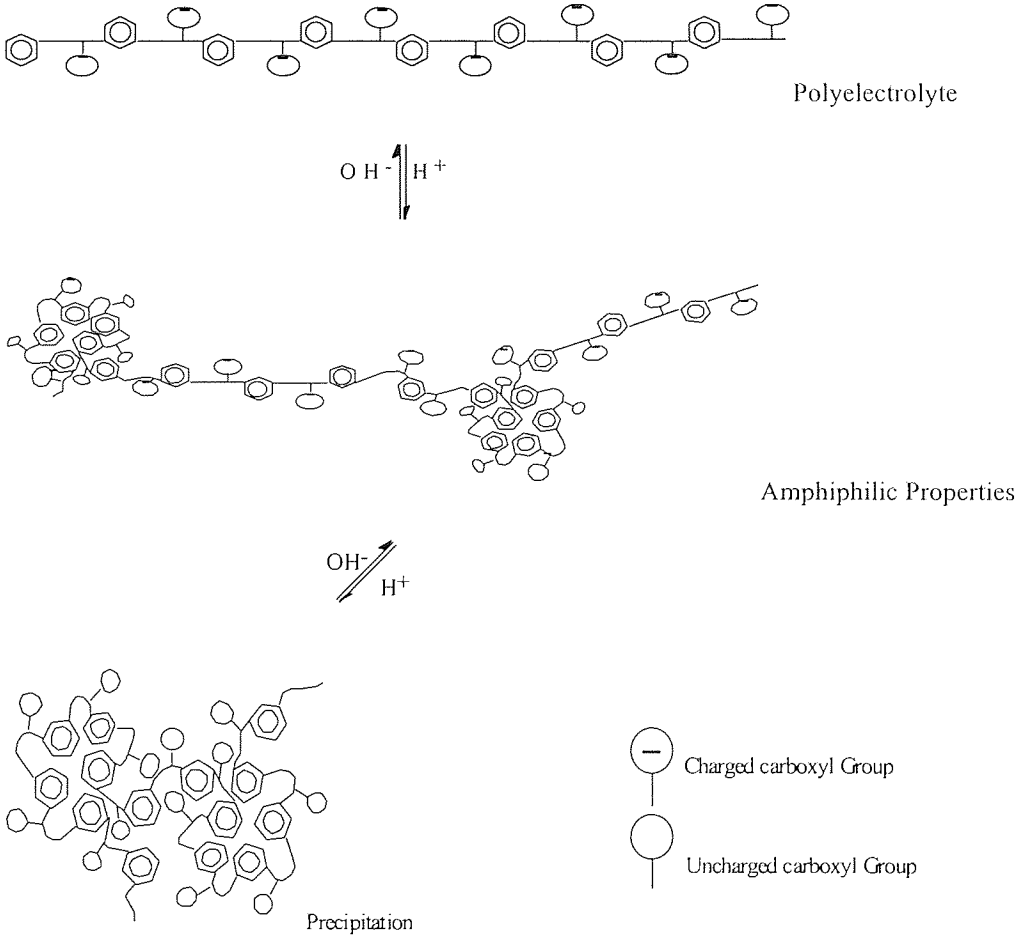
Two types of secondary structure have been well documented. Firstly, certain poly (amino acids) such as poly (glutamic acid) exhibit a helical structure at low degrees of ionisation. This structure becomes increasingly unstable with increases in pH and eventually polyglutamic acid adopts a random coil structure when the degree of ionisation increases beyond 0.4[167].

Secondly several synthetic hydrophobically modified polyacids are known which exist in a tightly coiled polysoap conformation at low degrees of ionisation (typically < 0.2)[61,164-168]. This so called hypercoiled structure is stabilised by cohesive intramolecular Van der Waals forces between the hydrophobic moieties within the polymer. As the degree of ionisation is increased the conformation changes to a random statistical coil and then to an extended rod like conformation as the electrostatic repulsion between the carboxylate ions overcomes the hydrophobic attraction of the apolar moieties. In the hypercoiled conformation there is some association of carboxyl groups through hydrogen bonding and the remaining carboxylate ions are drawn closer together increasing the local electrostatic potential[164,165]. Thus, the apparent pKa of a hydrophobically associating polyacid is relatively higher at low degrees of ionisation than it would be in a non associating polymer.

The potentiometric titrations of the polymers synthesised in this thesis suggested that at low degrees of ionisation poly (ornithine *iso*-phthalamide) and poly (lysine *iso*-phthalamide) exist in compact states stabilised by hydrophobic association of the *iso*-phthaloyl groups. Thus, the apparent pKa values of these polyacids at low degrees of ionisation are disproportionately high based on a simple linear charge distribution model. In order for a polymer to exhibit a hypercoiled conformation it must contain both weakly charged hydrophilic groups and asymmetrically positioned hydrophobic groups coupled with a degree of flexibility within the polymer backbone that allows separation of these two distinct moieties into distinct environments.

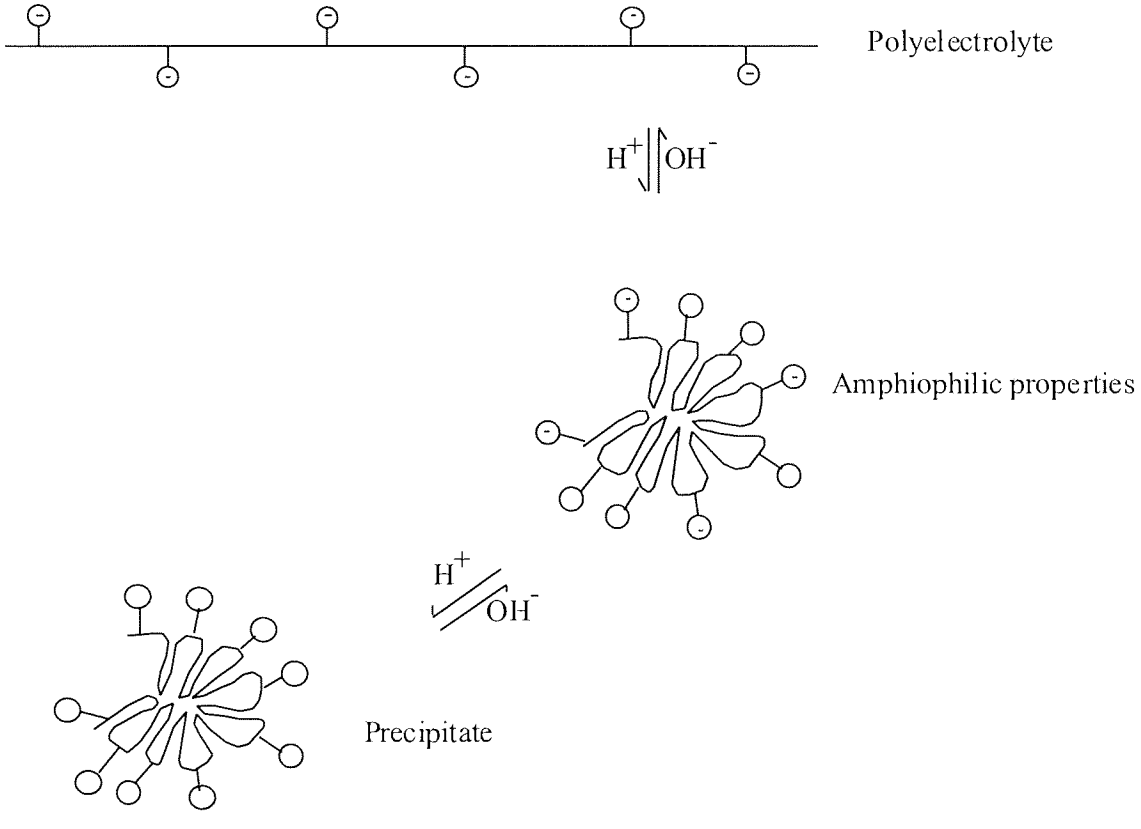
Upon loss of charge the hydrophobic groups collect within the core of the collapsing coil whilst the remaining charged hydrophilic groups tend to locate at the outer surface of the coil. Since this is an intramolecular association process then it could be assumed that there may be a critical molecular weight of polymer below which no association will occur. This is supported by the work of Tonge who found that poly (styrene maleic acid) with a molecular weight less than 2000 Da did not hypercoil whereas higher molecular weight samples did so readily. Thus poly (*iso*-phthalamides) of lysine and ornithine appear to under go a conformational transition as the weak carboxylic acid groups of the amino acid moiety are neutralised. This transition is driven by the association of the hydrophobic *iso*-phthaloyl groups asymmetrically positioned within the polymer backbone (Fig. 6.5).

Fig. 6.5 Proposed conformational change of poly (lysine iso-phthalamide) with pH.



This conformational transition is identical to that seen in poly (lysine 1,3 sulphonamide)[61] at low degrees of ionisation. The poly (dodecanamides) of lysine and ornithine also appear to undergo such a transition apparently despite any hydrophobic asymmetry. It is proposed that upon loss of charge poly (lysine dodecanamide) contracts as expected for a normal polyelectrolyte but the relatively long olefinic chain within the polymer backbone permits the formation of an asymmetric hydrophobic "tail" within the polymer backbone. Thus co-operative association of these hydrophobic tails pulls the polymer into a hypercoiled conformation (Fig. 6.6).

Fig. 6.6 Proposed conformational change of poly (lysine dodecanamide) and poly (ornithine dodecanamide) in response to changes in pH.



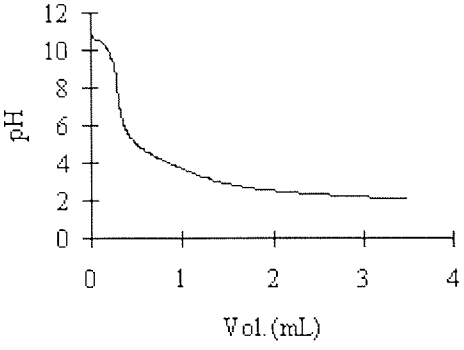
It can be seen from the graphs of variation in pH (graphs 6.11, 6.14 and 6.17) and variation in apparent pKa (graphs 6.12, 6.15 and 6.18) with degree of ionisation that the onset of the conformational transition in the poly (dodecanamides) takes place at higher degrees of ionisation than in the poly (*iso*-phthalamides).

This indicates increased hydrophobicity in the aliphatic polyamides relative to the aromatic poly (*iso*-phthalamides) which must in turn be balanced by a higher degree of ionisation to prevent coiling. It may be possible to control the degree of ionisation at which the polyamide begins to collapse by varying the length of the aliphatic acid chloride although it is to be expected that a minimum length would be required for the conformational transition to be energetically favourable. Additionally increasing the length of the chain would ultimately lead to the polyamide being insoluble at all values of pH.

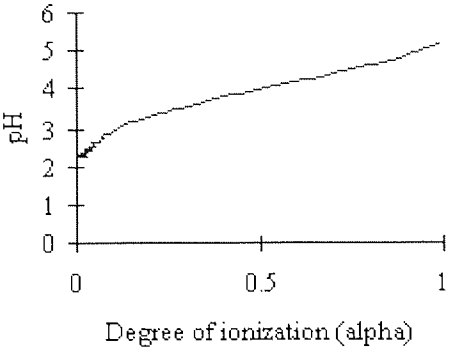
In the case of poly (lysine diethylmalonamide) the hydrophobicity is distributed symmetrically about the backbone of the polymer and thus precludes the formation of a hydrophobic core. The polymer titrates as a normal polyacid with loss of charge, eventually precipitating from solution (graphs 6.7-6.9).

One would expect high molecular weight polyamides of phenylmalonic and phenylglutaric acid to show increased hydrophobic bonding because of the increased asymmetry of the hydrophobic groups and the increased flexibility of the polymer backbone. These factors should lead to a conformational transition at a higher pH, i.e. at higher degrees of ionisation than in the poly (*iso*-phthalamides). In the case of poly (phenylglutamide) and poly (phenylmalonamide) the absence of a second equivalence point (Graph 6.19) is attributed to the relatively low molecular weight of these polymers (Sec. 5.6, Table 5.15). The variation in pH and pKa with degree of ionisation (graphs 6.20 and 6.21) also indicate the absence of any secondary structure at low degrees of ionisation.

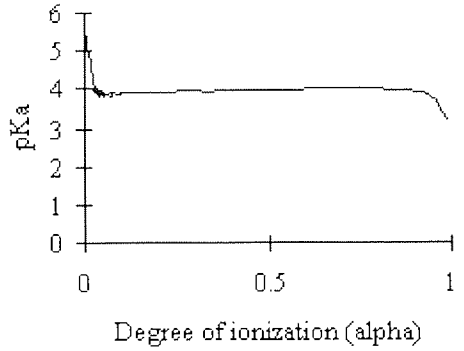
Graph 6.19 Variation in pH with volume of added titrant for poly (lysine phenylglutamide).



Graph 6.20 Variation in pH with degree of ionization of poly (lysine phenylglutamide).



Graph 6.21 Variation in apparent pKa with degree of ionization of poly (lysine phenyl glutamide).



7. CHAPTER 7

DISCUSSION

The aim of this project was to investigate methodologies for the synthesis of pH responsive polymers for biomedical applications. The synthesis of polymers containing hydrophilic, weakly charged carboxyl groups and hydrophobic groups was investigated. Potential biomedical applications of polymers bearing weakly charged carboxyl groups include enteric coatings (which rely on dissolution of the polymer in the charged state), hydrosoluble drug/polymer conjugates capable of precipitating at, and thus targeting, areas of low pH (a salient feature of many solid tumours), or micelle forming polymer/drug conjugates. Micellular polymer-drug conjugates can increase the aqueous solubility and specificity of hydrophobic drugs. In such systems, the drug contributes to the hydrophobicity of sections of a hydrophilic polymer, resulting in the assumption of a micellular conformation. Hydrophobically modified polyelectrolytes may also adopt amphiphilic structures upon partial loss of charge. Hydrophobic bonding within the polymer can result in the formation of a distinct lipophilic core, surrounded by the remaining charged groups. This phenomenon is known as hypercoiling. It may be possible to solubilise drugs within these intramolecular micelles. Continued neutralisation of the charged groups results in precipitation of the polymer. Desirable features of polymers intended for biomedical application were perceived to be: low systemic toxicity; biodegradability; controllable response to pH and ease of synthesis.

To satisfy the first three conditions, multifunctional amino acids were considered to be a suitable starting point. Condensation of diamino acids, with diacyl chlorides, through formation of non-peptidic amide bonds can be used to produce polymers bearing the required functional groups. Variation of the diacid chloride, or partial esterification of the carboxyl group of the diamino acid can be used to manipulate the hydrophobic/hydrophilic balance. By such structural changes, the effect of changes in pH on the aqueous solubility and conformational characteristics of the polymer can be tailored to specific applications. The polymers were wholly, or at least partially, based on naturally occurring metabolites, food additives or pharmacologically acceptable starting materials. These structural analogues of proteins can be referred to as pseudo-proteins. Use of such pseudo-proteins *in-vivo* would reduce possible immunological response from the host by avoiding the primary peptide sequence seen in proteins.

Since the polymers were based on natural metabolites, it was to be expected that the degradation products would show low toxicity. In addition, the rate of degradation was expected to be higher than that of simple polyamides due to the electron withdrawing effect of the carboxyl group adjacent to alternate amide linkages. Cleavage of these hydrolytically sensitive linkages would result in low molecular weight soluble degradation products which could be cleared through the kidneys.

Once the required features of the polymers had been determined, a suitable synthetic strategy was required. The polyfunctional nature of diamino acids meant that traditional high temperature melt polymerisation could not be used to synthesise functional polyamides of the type described in this thesis. Under the conditions of such a reaction, the relative differences in reactivity of the carboxyl and amine functions would be insufficient to ensure the production of linear polymer. Several low temperature solution methods are available, however, these methods tend to be slow and still require protection of groups that may potentially compete with the amine in the reaction with the carboxylic acid. Solution methods generally require exact stoichiometric equivalence meaning high purity, solubility of reagents and solubility of the resulting polymer within the solvent system for high molecular weights to be attained.

Interfacial polymerisation was initially selected as the synthetic method for condensing diamino acids, via their α- and ω-amino groups, with diacid chlorides. This technique has been used extensively since it was developed in the 1940s for the production of Nylon-type polyamides. Under the conditions of interfacial synthesis a diamine and acid acceptor in aqueous solution are stirred rapidly with an organic solution of a diacid chloride. The diamine usually has an appreciable equilibrium partition coefficient in the organic solvent. Once the diamine and the diacid chloride come into contact on the organic side of the interface rapid and irreversibly reaction takes place. Since reaction between the diamine and diacid chloride takes place on the organic side of the interface, hydrolysis of the diacid chloride is precluded.

The interfacial process allows the direct condensation of diamino acids with aromatic and certain aliphatic diacid chlorides, without protection of the α-carboxyl group. The resulting ABAB type polyamides contained carboxyl groups pendant to the main polymer chain. If non-esterified diamino acids were used, then the polymer remained dissolved in the aqueous phase at the end of the reaction. In addition the diamino acid would be in the carboxylate form under the basic conditions of the reaction. Consequently the equilibrium partition coefficient of diamino acid moiety in the organic solvent would be considerably lower than that of an uncharged diamine or an esterified diamino acid. Use of the aliphatic diacid chloride series was restricted by the increasing hydrolytic sensitivity of the shorter members of the series coupled with their increased solubility within the aqueous phase. It was also found that diamino acids with fewer methylene units than ornithine (three) were not suitable for interfacial polycondensation with diacid chlorides. This was ascribed to the lower nucleophilicities of these diamino acids coupled with their low equilibrium partition coefficients in the organic solvents. Production of linear functional polymers by the interfacial method was possible because of the rapidity of the main reaction between amine and acid chloride. The main reaction effectively outpaced the slower reaction between acid chloride and carboxyl groups which would result in the formation of in-chain aromatic or crosslinking aliphatic/aromatic mixed anhydride groups.

It was found that the molecular weight of interfacially synthesised poly (ornithine *iso*-phthalamide) was dependant on a number of experimental variables, although it was clear that these variables had a common consequence i.e., increased contact between the diamine and the diacid chloride. Thus, increasing the stirring efficiency, increasing the concentration of the diamino acid in the aqueous phase, excess diamine and added sodium chloride all resulted in an increase in the final molecular weight of the polymer. These are all factors which would be expected to increase the equilibrium partition coefficient of the diamine in the organic phase.

Interestingly, increasing the concentration of the diacid chloride in the organic phase or using an excess of diacid chloride also increased the molecular weight of the final polymer. In view of the limited availability of the diamine in the organic phase, it was postulated that the site of reaction may have shifted depending on the nature of the diamine moiety.

In general, the rate of reaction of two species in an interfacial reaction is governed principally by the availability of the two species at the locus of polymerisation. The locus of polymerisation is related to the equilibrium partition coefficients of the reacting species in the two immiscible solvents. If the amine-containing species is uncharged (as a simple diamine would be) then it has an appreciable equilibrium partition coefficient in the organic solvent and the locus of polymerisation approaches the organic side of the interface. If, however, the amine-containing species is charged under the conditions of the reaction (as a non-esterified amino acid would be) then it has a low equilibrium partition coefficient in the organic phase and the locus of polymerisation approaches the aqueous side of the interface. In this situation the diacid chloride was more exposed to hydrolysis. In the polymerisation of *iso*-phthaloyl chloride and ornithine an excess of diacid chloride may have offset any loss of acid chloride due to hydrolysis.

The apparent shift in locus of polymerisation was supported by the production of two distinct polymers rather than a single copolymer when *iso*-phthaloyl chloride was reacted interfacially with a mixture of lysine and lysine ethyl ester. An organic solvent swollen precipitate was produced rapidly on stirring and a second precipitate was obtained at the end of the reaction on acidification of the aqueous phase. FT-IR analysis showed the initial precipitate to contain predominantly lysine ethyl ester as the diamine moiety whist the second precipitate contained predominantly lysine as the diamine moiety. Similar results were obtained when *iso*-phthaloyl chloride was reacted interfacially with a mixture of lysine or ornithine and hexamethylene diamine in that an insoluble product precipitated during the course of the reaction and a soluble product was retained in the aqueous phase.

If, however, the aqueous solution of lysine or ornithine was added to the organic solution of *iso*-phthaloyl chloride prior to the hexamethylene diamine then a different outcome was observed. The supply of diamino acid was initially limited to 50% of the diacid chloride concentration followed by addition of an equivalent amount of hexamethylene diamine to achieve a stoichiometric balance of amine and acid chloride functionalities. Under these conditions a single insoluble polymer was produced. FT-IR analysis indicated that in addition to the expected amide bonds, aromatic anhydride linkages were also present.

It is postulated that following addition of the initial substoichiometric amount of diamino acid some hydrolysis of acid chloride terminal groups to aromatic carboxylic acid groups occurred. Subsequent condensation of those acid groups with additional acid chloride groups in the absence of additional diamine would result in formation of anhydride linkages. It is unlikely that anhydride formation would be exclusively between acid chloride and aromatic carboxyl end groups. It is probable that the more reactive aliphatic anhydride linkages formed by reaction of acid chloride groups with the pendant carboxyl groups were cleaved soon after formation under the basic conditions of the reaction. On addition of the hexamethylene diamine, the polymerisation continues and precipitation of the polymer protects the remaining anhydride groups from hydrolysis. Under these conditions the molecular weight of the final polymer would be low.

The range of molecular conformations attainable by a hydrosoluble polymer and thus the apparent pKa of the polymer may be influenced by inclusion of asymmetric hydrophobic moieties within or pendant to the polymer backbone. Formation of hydrophobic microdomains in response to a decrease in the degree of ionisation of the weakly charged hydrophilic carboxylate groups can lead to the formation of a tightly hypercoiled system, stabilised by hydrophobic bonding. In such a conformation, the remaining carboxylate groups along the backbone of the polymer are held in close proximity to each other and experience a higher local charge density than would be predicted by a simple linear distribution of charge along the polymer.

The effect of this increase in local electrostatic field is to reduce the ease of ionisation of the carboxyl groups i.e. increase the pKa of the carboxyl groups. It was demonstrated that structural modification of lysine and ornithine containing polyamides allows modification of the apparent pKa values and pH of minimum aqueous solubility of the polymers. Thus the pKas of poly (lysine *iso*-phthalamide) and poly (lysine dodecamide) for example are higher than that of poly (lysine diethylmalonamide) at low degrees of ionisation.

If the hydrophobicity is symmetrically distributed around or within the backbone of the polymer, e.g., poly (lysine diethylmalonamide), then it is generally not possible for the polymer to adopt a conformation which allows the hydrophobic and hydrophilic segments to segregate into separate domains on partial neutralisation. The polymer behaves as a normal poly acid displaying a progressive increase in pKa with degree of ionisation. This results from the increase in electrostatic field as successive carboxyl groups are ionised. The electrostatic potential experienced causes the polymer chain to expand. The chain expansion is accompanied by a partial reduction in the electrostatic potential in the absence of any hydrophobic association. Thus the chain expands until the charge repulsion between the carboxylate groups balances the natural tendency of the polymer to assume a random conformation in solution due to Brownian motion. In the case of poly (lysine *iso*-phthalamide), poly (ornithine *iso*-phthalamide), poly (lysine dodecanamide) and poly (ornithine dodecanamide), hydrophobic association of either the asymmetric *iso*-phthalamide groups or the long aliphatic chains resists expansion of the polymer chain at low degrees of ionisation. The carboxyl groups thus experience a relatively large electrostatic field and thus display unusually high pKas. The so called hypercoiling phenomenon is displayed by the structurally related poly (lysine1,3-benzene disulphonamide) and a number of vinyl copolymers such as poly (styrene maleic acid) and is characterised by a pH dependent amphiphilic state.

Modification of the hydrophilic/hydrophobic balance would allow the pH of onset of amphiphilic properties to be tailored. Such modifications would also effect the pH of minimum aqueous solubility by changing apparent pKa of the polymer. The range of pKa values and pH of minimum aqueous solubility so far achieved is well below that of physiological pH at approximately 7.4 and is too low to be used as a possible trigger for precipitation in the relatively acidic environment of a solid tumour, typically in the range 6.9-7.2. This range may be accessible by controlled copolymerisation of lysine and lysine ethyl ester with *iso*-phthaloyl chloride. Hydrophobic modification of cellulosic and vinyl polymers through partial esterification of carboxyl functions has been used extensively in the manipulation of aqueous solubility characteristics of polymers for enteric coatings.

Manipulation of the hydrophilic/hydrophobic balance was attempted by utilising different diacyl moieties and through variation of the ratio of free carboxyl to esterified α-carboxyl groups on the lysine moiety. From the literature it is evident that previous workers have used both free lysine and lysine ethyl ester to produce lysine containing polyamides. The free acid derivatives are regenerated from the esterified derivatives by mild hydrolytic treatment. Two problems were envisaged with this approach to the synthesis of polyamides containing varying but controlled proportions of free and esterified lysine moieties. Firstly a reduction in molecular weight of the polyamide was anticipated because of backbone scission occurring during the hydrolytic cleavage of the ester functions. The higher hydrolytic stability of the amide linkage relative to the ester linkage would reduce but not preclude this possibility. Secondly controlled cleavage of ester groups to give reproducible ratios of esterified to free α-carboxyl groups may have proven difficult. In order to circumvent this problem the direct synthesis of copoly (*iso*-phthalamides) of lysine and lysine ethyl ester was investigated.

In an ideal system the final ratio of lysine ethyl ester to lysine in the polymer would reflect (or at least approximate to) the initial diamine ratio in the monomer feed. The system should also allow the possibility of block polymer formation which may permit synthesis of biodegradable micelle forming polyamides.

In general, in an interfacial system factors such as relative solubility in the aqueous and organic solvents make such a requirement difficult. Increasing the equilibrium partition coefficient of a charged diamino acid such as lysine or ornithine in the organic phase would shift the locus of polymerisation through the interface and into the organic side of the interface with an associated increase in molecular weight. Choice of solvent, surfactants, salting out effects and temperature can be used to influence the solubilities of the diamines within the organic phase, but these factors cannot be applied independently to one diamine. Low availability of the lysine moiety within the organic phase explains the relatively low molecular weight of lysine containing polymers synthesised by the interfacial method. Single phase systems have been investigated for the production of lysine containing hetero-polymers. Typically such polymerisations require stabilised lysine derivatives such as N^{α},N^{ε}-bis-trimethylsilyated lysine ethyl ester because of the poor solubility of lysine ethyl ester in organic solvents.

The use of miscible mixed solvent systems as a rapid and simple technique to allow the copolycondensation of hydrophobic and hydrophilic diamine moieties with diacid chlorides was investigated. Several advantages over an interfacial technique were envisaged by using such a technique. These include increased contact between reagents, improved solubility of reagents in unmodified form in the solvent system, reduced polydispersity and use of environmentally friendly solvents.

In a single phase polycondensation, equilibrium partition coefficients will have no significance and the ratio of diamine moieties in the backbone will depend solely on the nucleophilicities of the amine functions. Since the relative reactivities of the amine functions are expected to be similar, copolyamides based on lysine and lysine ethyl ester could be synthesised with diamine ratios approximating those of the feed. Using mixed solvent systems overcomes the problem of solubility of the two monomers and acid acceptor in a single solvent. Amino acids and inorganic bases are poorly soluble in organic solvents but the amino acids are readily soluble in the ionised form in aqueous alkali. These advantages, however, are balanced by increased exposure of the diacid chloride and acid chloride oligomers to hydrolysis.

The single phase polycondensation technique offers an environmental and financial advantage in that chlorinated or aromatic solvents typically used in interfacial syntheses can be avoided. The method was successfully applied to the synthesis of polyamides from aromatic diacid chlorides and lysine and/or lysine ethyl ester.

When mixed aqueous solutions of lysine and lysine ethyl ester were reacted with *iso*-phthaloyl chloride in acetone, only one product was obtained and the solubility of that product, in the solvent system, could be varied with the ratio of free amino acid to esterified diamino acid. Poly (lysine-co-lysine ethyl ester *iso*-phthalamide) 1:1 was soluble in the solvent mixture whereas 1:3 was insoluble and precipitated as a gummy material throughout the course of the reaction. Poly (lysine ethyl ester *iso*-phthalamide) synthesised using the miscible mixed solvent method was found to be less polydisperse with equivalent or higher molecular weight (as determined by GPC) than that produced by an interfacial method. The polydispersity of poly (lysine ethyl ester *iso*-phthalamide) synthesised by the interfacial method was reduced by extraction of oligomers in acetone and it was reasoned that a lower molecular weight fraction was retained in the aqueous/acetone solvent at the end of the miscible mixed solvent reaction. A low molecular weight fraction tended to precipitate as a film at the liquid/air interface over a period of hours following precipitation of the main high molecular weight fraction. The slow precipitation of low molecular weight material may have occurred as a result of gradual evaporation of the more volatile acetone fraction of the solvent system.

In common with the interfacial process discussed previously, under specific conditions, i.e. when an aqueous solution of lysine ethyl or methyl ester was added slowly to a solution of *iso*-phthaloyl chloride in acetone, it was found that anhydride formation occurred. Since the α-carboxyl functions of the diamino acid were esterified and thus not available for reaction with the diacid chloride it is postulated that anhydride formation occurred principally between hydrolysed acid chloride groups and additional acid chloride. This was confirmed by FT-IR analysis which indicated the formation of aromatic rather than aliphatic anhydride linkages. The low molecular weight of the resultant polymer was assumed to be due to hydrolysis of acyl chloride terminated oligomers and the in-chain anhydride linkages under the alkali reaction conditions.

GPC analysis of the poly (lysine ethyl ester *iso*-phthalamide) samples produced under a variety of experimental conditions showed that highest molecular weight (corresponding to highest yield) were achieved with an equivalent of amine, acid chloride and acid acceptor. Added salt reduced the final molecular weight of the polymer presumably because of reduced phase miscibility.

A detailed study of the molecular weight distributions of poly (lysine *iso*-phthalamide) was not undertaken because of problems encountered in the study of poly (ornithine *iso*-phthalamide) synthesised by the interfacial method. Such a study would be necessary to confirm the validity of the technique for producing high molecular weight copolymers of lysine and lysine ethyl ester with *iso*-phthaloyl chloride. It is suggested that aqueous GPC using a system comparable to that used for protein analysis would be more appropriate for studying such polymers. The relatively high molecular weight of poly (lysine *iso*-phthalamide) produced using a miscible mixed solvent technique compared to that produced using an interfacial technique could be inferred by the lower amount of soluble material soxhlet extracted in acetone from the polymer produced using the miscible mixed solvent technique compared to that produced by the interfacial technique.

Aliphatic and non-conjugated aromatic diacid chlorides are less suited to polycondensation with diamines using miscible mixed solvents than are aromatic diacid chlorides because of their lower hydrolytic stability. In an interfacial reaction, higher molecular weight aliphatic acid chlorides such as dodecanedioyl dichloride, were successfully condensed with simple diamine and diamino acids. The long aliphatic chain reduces the equilibrium partition coefficient of the acid chloride in the aqueous phase and thus the rate of hydrolysis is reduced. As the length of the acid chloride is reduced its equilibrium partition coefficient in the aqueous phase increases as does its rate of abstraction. Morgan[1] states that in an interfacial reaction the effective rate of hydrolysis of the lower molecular weight acid chlorides is equivalent to their rate of abstraction into the aqueous phase.

In general, in a single phase mixed solvent system containing water no protection is afforded to even long chain diacid chlorides and hydrolysis may be to rapid to allow formation of high molecular weight material. It may be possible to synthesise higher molecular weight aliphatic polyamides using miscible mixed solvents if the phase ratio of the aqueous phase to the organic is reduced whilst maintaining the overall stoichiometric balance. Such a modification of the reaction conditions may also be beneficial to reactions with aromatic diacids. This possibility was not investigated.

The molecular weight distributions of the polymers were determined using gel permeation chromatography. The molecular weights are expressed as PEO/PEG equivalents and in view of the chemical dissimilarity between the synthesised polymer and PEO/PEG there may be considerable differences between the PEG equivalent molecular weights and the actual polymer molecular weights. The results are best viewed in a comparative manner and allow qualitative analysis of the effects of reaction variables on the relative molecular weights of the polymers synthesised. The long term reproducibility of the technique was unreliable hence comparison was only made between chromatograms run at the same time.

The short term reproducibility (over a period of a few days) between chromatograms of poly (ornithine *iso*-phthalamide) proved to be poor. In addition these samples had unusually large apparent polydispersities, sometimes in excess of 100. Whilst interfacially synthesised polymers often have broader molecular weight distributions than those produced by melt or solution polycondensation, the difference is usually a factor of 2 or 3 not 50. It is doubtful that the large polydispersity is a true reflection of the molecular weight distribution of the polymer sample. One of the main reasons for the apparently large polydispersites was the inclusion of oligomeric material along with the main peak when the molecular weight averages were calculated from the chromatogram. The molecular weight averages and polydispersisities of the main peak, reflecting the bulk of the material, may be more appropriate for a comparison of samples since low molecular weight material would be removed prior to any biomedical application. Alternatively the peak molecular weight would provide a useful comparison between the polymeric molecular weights.

Other possible reasons for a relatively large include interaction between the polymer and column, repulsion of polymer from the column or possible chelation of inorganic salts added to the mobile phase by the polymer. It is likely that a number of phenomena were occurring simultaneously creating a complex system. Polymers of esterified amino acids, specifically lysine ethyl ester were less problematic. However similar broadening of the molecular weight distribution has been noted when the poly (lysine *iso*-phthalamide) samples were run after samples of poly (lysine *iso*-phthalamide) indicating some kind of memory effect of the column. In view of this, it may be necessary to flush the column exhaustively after running polyamides of free amino acids prior to running new samples.

The pH range of minimum aqueous solubility and the pKa of the polymers synthesised makes them suitable for use as enteric polymers. The film formability and mechanical properties of enteric properties have been demonstrated to be important in determining the effectiveness of the enteric coat. Solvent cast films of poly (lysine *iso*-phthalamide) were extremely brittle and exhibit poor compatibility with several common plasticisers. Poor film formability of the Eudragit® series of enteric polymers (ethacrylate and ethacrylic acid copolymers) means that high plasticiser contents are often required for acceptable coating efficiency and performance. This requirement also makes such polymers poor candidates for the novel surface neutralisation coating method.

Poly (lysine ethyl ester *iso*-phthalamide) films also show poor compatibility with common plasticisers. The range of applications and processability of both polymers could be extended if a suitable plasticiser could be found. Whilst relatively small quantities of plasticiser had a considerable effect on the mechanical properties of poly (lysine ethyl ester *iso*-phthalamide) no improvement was seen within the range of plasticiser compatibility of poly (lysine *iso*-phthalamide). Poly (lysine *iso*-phthalamide) may be internally plasticised through esterification of the pendant α-carboxyl groups in an identical way to the tyrosine containing polarylates synthesised by Kohn *et al*[3]. The single phase miscible mixed solvent method would be suited to the inclusion of small amounts of plasticising esterified diamino acid moieties into a predominantly carboxyl bearing polyamide. Problems of permanence and compatibility could thus be avoided.

In addition to use as hydrosoluble drug carriers, methods for the synthesis of microparticles were investigated. A novel solvent extraction/solvent evaporation process was developed which allowed the production of microparticles from presynthesised polyamides. Examination of the microparticles revealed that the particles were spherical with a honeycombed internal structure and a porous surface. The formation of such an internal architecture was noted by previous workers using related systems for the synthesis of microspheres and is believed to arise as a result of entrapment of dichloromethane (a poor solvent for the polymer) as the DMSO is extracted into the aqueous phase. The subsequent evaporation of the dichloromethane during the final drying stage leaves bubbles within the microspheres. Modification of the reaction conditions and the ratio of DMSO to dichloromethane could allow control of the internal structure of the microparticles from microsphere to microcapsule and on the porosity of the surface. The release characteristics of the microparticles could then be tailored for specific applications.

The synthesis of pH responsive polyamides, intended for biomedical application, was investigated. Interfacial synthesis was used to produce polymers derived from tri-functional naturally occurring diamino acids and aliphatic or aromatic acid chlorides. Conditions were identified for the synthesis of polymers, bearing weakly chargeable carboxylic acid groups, with suitably high molecular weights for application as hydrosoluble drug delivery vehicles. The molecular weight of poly (ornithine *iso*-phthalamide) increased as the equilibrium partition coefficient of the diamino acid, ornithine, was increased in favour of the organic phase. An increase in the equilibrium partition coefficient, with respect to the organic phase, was achieved by increasing stirring speed, increasing polarity of the organic solvent (whilst retaining a two phase system), increasing the concentration of the aqueous phase in terms of diamino acid or by adding inorganic salts. The increase in molecular weight is attributed to a shift in the locus of polymerisation from the aqueous side of the interface towards the organic side of the interface with an associated reduction in hydrolysis of the *iso*-phthaloyl chloride. In addition, a single phase mixed solvent technique was investigated as a method for synthesising copolymers of free and esterified diamino acids with aromatic diacid chlorides.

The synthesis of such copolymers would allow modification of the balance of hydrophobicity and hydrophilicity of the resulting copolymers. The ability to manipulate the balance of hydrophobicity and hydrophilicity of a weakly charged polymer allows the aqueous solubility and conformational characteristics and their dependence on the pH to be tailored to suit specific applications.

When lysine ethyl ester dihydrochloride was polymerised with *iso*-phthaloyl chloride it was found that in common with single solvent solution polymerisations, the highest molecular weights were achieved with an equivalence of reagents. Added inorganic salts caused a reduction in the molecular weight of the polymer. It was found that copoly (*iso*-phthalamides) of lysine or ornithine and hexamethylene diamine or lysine ethyl ester could be synthesised and that the solubility of the product, in the solvent system, could be varied with the ratio of diamino acid to esterified diamino acid or diamine. Thus by reducing the relative amount of diamino acid, the hydrophobicity of the of the co-polyamide could be increased and the solubility in an aqueous system could be reduced. The ability to increase the hydrophobicity of a polymer based on a particular diacid chloride and diamino acid such as poly (lysine *iso*-phthalamide) by inclusion of controlled amounts of lysine ethyl ester was thus possible using a single phase miscible mixed solvent technique.

8. CHAPTER 8

CONCLUSIONS AND SUGGESTIONS FOR FUTURE WORK

A number of functional polyamides bearing carboxyl groups have been synthesised in this thesis using interfacial synthesis and a novel miscible mixed solvent single phase polycondensation technique. It has been demonstrated that the pKa and solubility characteristics can be manipulated through structural modification of the polymer. Such structural modification can be achieved by utilising alternative diacyl chloride moieties to condense the diamino acid moieties or by copolymerisation of the diacyl moiety with diamines, diamino acids and diamino acid esters. Further control over the hydrophobic hydrophilic balance of the polymer could then be achieved through variation of the length of the ester chains. Further investigation of this variation will form the basis of a final year undergraduate research project. The structural modification of the steric environment of the carboxyl group to influence pKa will ultimately be limited by factors such as solubility of the polymer. The pH range over which conformational change, dissolution or precipitation occurs may be extended by utilising alternatively weakly charged moieties such as sulphonic, phosphoric or boric acid functions. These may be derived from alternative polyfunctional diamino moieties or through conversion of other functional groups.

Interfacial methods are not suited to the synthesis of copolymers from diamino moieties with largely different equilibrium partition coefficients since the availability of one of the diamino moieties will inevitably be lower than the other. A single phase system offers advantages in this respect but may be difficult to achieve in practice because of the differing solubilities of the diacyl chloride and diamino acid/acid ester moieties. A miscible mixed solvent system consisting of acetone and water was used to overcome these solubility problems and produced homopolymers of *iso*-phthaloyl chloride and lysine ethyl ester with comparable molecular weights to the interfacial method. Such a single phase system is not suitable for synthesis of aliphatic polyamides because of increased hydrolysis of the diacyl chloride compared to the interfacial method.

A series of lysine/lysine ethyl ester *iso*-phthalamide copolymers is currently being evaluated for biocompatibility at the Cancer Research Centre in the Queen Elizabeth hospital in Birmingham. Initial results suggest that the polymers are well tolerated *in-vitro*. A study of the biodegradation profiles of the polymers will follow. It was envisaged that pH triggered precipitation could be used as a method of visualising the local pH within the interstitium of solid tumours. Solid tumours frequently display a leaky vasculature which can be exploited as a method of passively targeting macromolecules to these areas since in most normal tissue macromolecules above a critical molecular weight do not cross the endothelium and enter the interstitium. The enhanced permeability of the tumour vasculature is complimented by a reduced lymphatic drainage in the tumour interstitium which results in the retention of macromolecules. Solid tumours are also characterised by poor vasculature and high interstitial pressure (which contributes to the poor vasculature by causing the collapse of blood vessels). As a result oxygen deprivation can lead to the formation of necrotic foci and a build up of lactate. Many solid tumours thus have low inter and intracellular pHs. Selective uptake of a weak polyacid to the tumour interstitium could be achieved using the enhanced permeability and retention effect. Once in the tumour interstitium local pH variations could be probed using the precipitation of the fluorescently labelled polyacid. Such a device would have obvious applications as an imaging or drug delivery device and may offer a method for tumour encapsulation. The use of pH responsive polymers as precipitative targeting devices is currently being investigated as part of a two year BBSRC funded postdoctoral research program. In addition to this novel application the polymers synthesised in this thesis have pKa values and solubility characteristics which make them suitable for application as enteric coatings. The ability to modify the pKa and pH of minimum solubility would allow specific areas of the GI tract to be targeted.

The polymers synthesised in this thesis have been characterised principally by infra red spectroscopy and GPC. Infra red spectroscopy allowed rapid conformation of the main functional groupings within the resulting polymers, for example, the presence of amide linkages within the backbone and the presence of ester functions in the side chains. The presence of anhydride groups in polymers synthesised under specific conditions was also identified.

It is intended that the polymers will be further characterised by ^{1}H and ^{13}C NMR to determine the arrangement of triads within the polymer backbone. This will be coupled with 2 dimensional NMR analysis of the through space interactions of the polymer. This information can be applied to molecular modelling and thus used to determine secondary structures within the polymer. ^{1}H NMR will be used to quantify the ratio of ester to free acid groups in the lysine/lysine ethyl ester *iso*-phthalamide copolymers.

The enhanced permeability and retention effect is dependent on the molecular weight of the macromolecule intended for passive delivery. Thus, an accurate method of molecular weight determination and a knowledge of the molecular weight distribution of the polymer is essential since low molecular weight fractions may be unselectively taken up by normal tissue and particularly high molecular fractions may be excluded from uptake even through the relatively leaky tumour vasculature. Ideally fractions of narrow polydispersity will be used. GPC analysis of the poly (ornithine *iso*-phthalamide) polymers described in Ch 5 proved to be problematic in terms of both long and short term reproducibility. Recent developments in polar GPC systems of the type used to characterised the proposed polymers have been made at RAPRA technology. The suitability of these new systems for characterisation of the polymers proposed will be investigated. In addition, a vapour pressure micro osmometer will soon be in place within the department of chemical engineering and applied chemistry at Aston university and will offer an alternative method for molecular weight determination.

The polymers of the type proposed will only be useful as pH microprobes providing that they precipitate over a narrow pH range and that this range can be determined accurately prior to their use in either an *in-vitro* or an *in-vivo* model. Thus the pKa and pH of minimum solubility of each of the polymers will be determined in aqueous solution and in the biological fluid used in the *in-vitro* model.

A comprehensive surface chemistry laboratory has now been established within the department of chemical engineering and applied chemistry at Aston university. A wide range of dynamic techniques are available for the analysis of the surfactive properties of macromolecules. These techniques, including spinning drop tensiometer and pulsating bubble surfactometer allow accurate determination of surface and interfacial tension. Since hypercoiling polymers exhibit an amphiphilic stage in their transformation from extended to hypercoiled conformation, these techniques could be used to demonstrate the pH dependent surfactant behaviour characteristic of hypercoiling polymers.

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10. APPENDIX A

SUMMARY OF REACTION CONDITIONS FOR INTERFACIAL AND MISCIBLE MIXED SOLVENT SINGLE PHASE POLYCONDENSATIONS.

Table A1. Summary of single phase polycondensation reactions of *iso*-phthaloyl chloride with lysine ethyl ester. 2HCl using miscible mixed solvents.

Experimental variable	Sample code	Experimental conditions	Yield
general Method magnetic stirrer bar time of reaction 1hr	ME 3.1	[*iso*-phthaloyl chloride] 0.2M (50mL) [lysine ethyl ester.2HCl] 0.2M (50mL) [potassium carbonate] 0.7M rapid addition of organic phase	74%
[acid acceptor]	ME 3.2.1	[potassium carbonate] 0.50M	81%
	ME 3.2.2	[potassium carbonate] 0.55M	83%
	ME 3.2.3	[potassium carbonate] 0.60M	78%
	ME 3.2.4	[potassium carbonate] 0.65M	83%
	ME 3.2.5	[potassium carbonate] 0.70M	80%
	ME 3.2.6	[potassium carbonate] 0.75M	90%
	ME 3.2.7	[potassium carbonate] 0.80M	77%
	ME 3.2.8	[potassium carbonate] 0.85M	90%
reaction Time	ME 3.3.1	1/2 hr	76%
	ME 3.3.2	1 hr	76%
	ME 3.3.3	1 1/2 hr	81%
added Salt	ME 3.4.1	1g added KCl	75%
	ME 3.4.2	2g added KCl	68%
	ME 3.4.3	3g added KCl	62%
organic Solvent	ME 3.5	THF	98% (with overnight precipitate)
combined volume 1 l	ME 3.6.1	[potassium carbonate] 0.8M	72%
	ME 3.6.2	(overnight precipitate)	~10%
combined volume 0.5 l	ME 3.6.2	[potassium carbonate] 0.8M	84%
overhead Stirrer	ME 3.6.3	[potassium carbonate] 0.6M	65%
second phase addition	ME 3.7.1	rapid addition of organic phase	70%
	ME 3.7.2	rapid addition of aqueous phase	72%
	ME 3.7.3	slow addition of organic phase	71%
	ME 3.7.4.1	slow addition of aqueous phase	26%
	ME 3.7.4.2	treated with conc. H_2SO_4	

Table A2. Summary of single phase polycondensation reactions of *iso*-phthaloyl chloride with lysine methyl ester using miscible mixed solvents.

Experimental variable	Sample code	Experimental conditions	Yield
second Phase addition	ME 3.8.1	rapid addition of organic phase	73%
magnetic stirrer bar	ME 3.8.2	rapid addition of aqueous phase	75%
time of reaction 1hr	ME 3.8.3	slow addition of organic phase	20%

Table A3. Summary of single phase polycondensation reactions of *iso*-phthaloyl chloride with non esterified diamines using miscible mixed solvents.

Diamine	Sample code	Experimental conditions	Yield
lysine.HCl magnetic stirrer bar time of reaction 1hr	ME 3.9	[*iso*-phthaloyl chloride] 0.2M (50mL) [lysine.HCl] 0.2M (50mL) [potassium carbonate] 0.5M rapid addition of organic phase to aqueous phase	87%
ornithine.HCl magnetic stirrer bar time of reaction 1hr	ME 3.10	[*iso*-phthaloyl chloride] 0.2M (50mL) [ornithine.HCl] 0.2M (50mL) [potassium carbonate] 0.5M rapid addition of organic phase to aqueous phase	84%
hexamethylene diamine.2HCl magnetic stirrer bar time of reaction 1hr	ME 3.11	[*iso*-phthaloyl chloride] 0.2M (50mL) [hexamethylene diamine.2HCl] 0.2M (50mL) [potassium carbonate] 0.5M rapid addition of organic phase to aqueous phase	91%

Table A4. Summary of single phase polycondensation reactions of *iso*-phthaloyl chloride with lysine.HCl and additional diamines using miscible mixed solvents.

Diamines	Sample code	Experimental conditions	Yield
lysine.HCl and lysine ethyl ester.2HCl ratio 1:1	ME 3.12.1	[*iso*-phthaloyl chloride] 0.2M (50mL) [lysine.HCl] and [lysine ethyl ester.2HCl] 0.1M [potassium carbonate] 0.7M	81%
lysine.HCl and lysine ethyl ester.2HCl ratio 3:1	ME 3.12.2	[*iso*-phthaloyl chloride] 0.2M (50mL) [lysine.HCl] 1.5M [lysine ethyl ester.2HCl] 0.5M [potassium carbonate] 0.7M	84%
lysine.HCl and hexamethylene diamine.2HCl ratio 1:1	ME 3.13	[*iso*-phthaloyl chloride] 0.2M (50mL) [lysine.HCl] and hexamethylene diamine.2HCl] 0.1M [potassium carbonate] 0.7M	72%

Table A5. Summary of single phase polycondensation reactions of diethylmalonyl chloride with selected diamines using miscible mixed solvents.

Diamine	Sample code	Experimental conditions	Yield
lysine ethyl ester.2HCl	ME 3.14	[diethylmalonyl chloride] 0.2M (50mL) [lysine ethyl ester.2HCl] 0.2M (50mL) [potassium carbonate] 0.6M	69%
lysine (free base)	ME 3.15	[diethylmalonyl chloride] 0.2M (50mL) [lysine] 0.2M (50mL) [sodium hydroxide] 0.4M	0%
lysine (free base)	ME 3.16.1	[diethylmalonyl chloride] 0.2M (50mL) [lysine] 0.2M (50mL) [potassium carbonate] 0.7M	78%
	ME 3.16.2	as above but 300mL each phase	74%

Table A6. Summary of single phase polycondensation reactions of 1,3-Benzene Di-Sulphonyl chloride with selected diamines using miscible mixed solvents.

Diamine	Sample code	Experimental conditions	Yield
lysine ethyl ester.2HCl	ME 3.17.1	[1,3-benzene di-sulphonyl chloride] 0.2M (50mL) [lysine ethyl ester.2HCl] 0.2M (50mL) [potassium carbonate] 0.7M	75%
lysine.HCl	ME 3.17.2	[1,3-benzene di-sulphonyl chloride] 0.2M (50mL) [lysine.HCl] 0.2M (50mL) [potassium carbonate] 0.7M	27%

Table A7. Summary of interfacial polycondensation reactions of 1,3-Benzene Di-Sulphonyl chloride with selected diamines.

Diamine	Sample code	Experimental conditions	Yield
lysine ethyl ester.2HCl organic solvent $CHCl_3$ overhead stirrer at 2000r.p.m. for 30 minutes no temperature control precipitate on addition of hexane initial precipitate Soxhlet extracted with chloroform	ME 4.1.1.1 ME 4.1.1.2 ME 4.1.1.3	[1,3-benzene di-sulphonyl chloride] 0.2M (50mL) [lysine ethyl ester.2HCl] 0.2M (50mL) [potassium carbonate] 0.7M	63% 29%
lysine.HCl organic solvent $CHCl_3$ overhead stirrer at 2000r.p.m. for 30 minutes no temperature control	ME 4.1.2	[1,3-benzene di-sulphonyl chloride] 0.2M (50mL) [lysine.HCl] 0.2M (50mL) [potassium carbonate] 0.7M	23%

Table A8. Summary of interfacial polycondensation reactions of *iso*-phthaloyl chloride with lysine ethyl ester.2HCl.

Experimental variable	Sample code	Experimental conditions	Yield
overhead stirrer (2000r.p.m.) 250mL baffled beaker organic solvent CCl_4	ME 4.2.1	[*iso*-phthaloyl chloride] 0.2M (50mL) [lysine ethyl ester.2HCl] 0.2M (50mL) [sodium hydroxide] 1.6M	0% before acidification of aqueous phase 66% (based on ester) or 73% (based on acid) after acidification.
overhead stirrer (2000r.p.m.) 250mL baffled beaker organic solvent CCl_4	ME 4.2.2.1 ME 4.2.2.2	[*iso*-phthaloyl chloride] 0.2M (50mL) [lysine ethyl ester.2HCl] 0.2M (50mL) [sodium carbonate] 1.6M hydrolysed sample	87% (unextracted)
overhead stirrer (2000r.p.m.) 250mL baffled beaker organic solvent CCl_4	ME 4.2.3.1 ME 4.2.3.2	*iso*-phthaloyl chloride] 0.2M (50mL) [lysine ethyl ester.2HCl] 0.2M (50mL) [potassium carbonate] 0.7M	72% coagulated and extracted in hot acetone for 30 minutes 17% acetone soluble material
overhead stirrer (2000r.p.m.) 250mL baffled beaker organic solvent CCl_4	ME 4.2.4.1 ME 4.2.4.2 ME 4.2.4.3	*iso*-phthaloyl chloride] 0.3M (300mL) [lysine ethyl ester.2HCl] 0.3M (300mL) [potassium carbonate] 1.2M soxhlet extracted with $CHCl_3$ $CHCl_3$ soluble	coagulated in acetone 84% 46% 53%
overhead stirrer (2000r.p.m.) 250mL baffled beaker organic solvent $CHCl_3$	ME 4.2.5.1 ME 4.2.5.2	*iso*-phthaloyl chloride] 0.2M (50mL) [lysine ethyl ester.2HCl] 0.2M (50mL) [potassium carbonate] 0.7M	initial precipitate 55% further precipitate on addition of hexane 37%
overhead stirrer (2000r.p.m.) 250mL baffled beaker organic solvent $CHCl_3$	ME 4.2.6	*iso*-phthaloyl chloride] 0.2M (50mL) [lysine ethyl ester.2HCl] 0.2M (50mL) [potassium carbonate] 0.7M	69% coagulate obtained on addition of initial solvent swollen gelatinous precipitate to acetone

Table A9. Summary of interfacial polycondensation reactions of *iso*-phthaloyl chloride with hydrophilic and hydrophobic diamines.

Diamines	Sample code	Experimental conditions	Yield
lysine.HCl and lysine ethyl ester.2HCl Ratio 1:1 reaction time 2hrs Organic solvent $CHCl_3$	ME 4.3.1 ME 4.3.2	*iso*-phthaloyl chloride] 0.2M (300mL) [lysine.HCl] and [lysine ethyl ester.2HCl] 0.1M (300mL) [potassium carbonate] 1.6M	initial precipitate 104% based on lysine ethyl ester acidified precipitate 42% based on lysine combined yield based on 1:1 ratio of diamines 74%
lysine.HCl and hexamethylene diamine.2HCl. Ratio 1:1 reaction time 2hrs organic solvent $CHCl_3$	ME 4.4.1 ME 4.4.2	*iso*-phthaloyl chloride] 0.2M (300mL) [lysine.HCl] and [hexamethylene diamine.2HCl] 0.1M (300mL) [potassium carbonate] 1.6M	initial precipitate 111% based on hexamethylene diamine acidified precipitate 37% based on lysine combined yield based on 1:1 ratio of diamines 72%
ornithine.HCl and hexamethylene diamine.2HCl. ratio 1:1 reaction time 2hrs organic solvent $CHCl_3$	ME 4.5 ME 4.6.1 ME 4.6.2 ME 4.6.3	*iso*-phthaloyl chloride] 0.2M (50mL) [ornithine.HCl] 0.2M (25mL) [potassium carbonate] 0.7M and [hexamethylene diamine.2HCl] 0.2M (25mL) [potassium carbonate] 0.7M addition of the first aqueous phase followed by rapid addition of the second aqueous phase half washed in boiling methanol half washed in boiling chloroform and acetone methanol washings	initial precipitate 73% based on 1:1 ratio of diamines no precipitate on acidification of aqueous phase.
As above with lysine.HCl	ME 4.7	As above with lysine.HCl	70% based on 1:1 ratio of diamines No precipitate on acidification of aqueous phase.

Table A10. Summary of interfacial polycondensation reactions of Itaconyl chloride with selected diamines.

Diamines	Sample code	Experimental conditions	Yield
lysine ethyl ester.2HCl organic solvent CCl_4 homogenised at 0°C for 15 minutes	ME 4.8	[itaconyl chloride] 0.2M (15mL) [lysine ethyl ester.2HCl] 0.2M (15mL) [sodium carbonate] 1M	59%
lysine ethyl ester.2HCl Organic solvent $CHCl_3$ homogenised at 0°C for 15 minutes	ME 4.9	[itaconyl chloride] 0.2M (15mL) [lysine ethyl ester.2HCl] 0.2M (15mL) [sodium carbonate] 1M	38%
lysine ethyl ester.2HCl CH_2Cl_2 organic solvent homogenised at 0°C for 15 minutes	ME 4.10	[itaconyl chloride] 0.2M (15mL) [lysine ethyl ester.2HCl] 0.2M (15mL) [sodium carbonate] 1M	36%

Table A11. Summary of interfacial polycondensation reactions *iso*-phthaloyl chloride with ornithine.

Experimental variable	Sample code	Experimental conditions
General Method homogenised at speed setting 1. with fine homogenising head and generator for 10 minutes at 0°C.		[*iso*-phthaloyl chloride] 0.25M (5mL) [ornithine.HCl] 0.2M (5mL) [sodium hydroxide] 1.6M
reaction time	ME 4.11.1 ME 4.11.2 ME 4.11.3	5 minutes 10 minutes 15 minutes
stirring Speed	ME 4.11.4 ME 4.11.5 ME 4.11.6	1 5 10
stirring Efficiency	ME 4.11.7 ME 4.11.8 ME 4.11.9 ME 4.11.10	fine head/fine generator ultrafine head/fine generator fine head/ultrafine generator ultrafine head/ ultrafine generator
concentration of aqueous phase	ME 4.11.11 ME 4.11.12 ME 4.11.13 ME 4.11.14 ME 4.11.15	[*iso*-phthaloyl chloride] 0.25M (5mL) [ornithine.HCl] 0.2M (5mL) [sodium hydroxide] 1.6M [ornithine.HCl] 0.25M (4mL) [sodium hydroxide] 2.0M [ornithine.HCl] 0.33M (3mL) [sodium hydroxide] 2.67M [ornithine.HCl] 0.5M (2mL) [sodium hydroxide] 4.0M [ornithine.HCl] 0.1M (1mL) [sodium hydroxide] 8.0M

Table A11. Continued.

Experimental variable	Sample code	Experimental conditions
concentration of organic phase		[ornithine.HCl] 0.2M (5mL)
		[sodium hydroxide] 1.6M
	ME 4.11.16	[*iso*-phthaloyl chloride] 0.25M (5mL)
	ME 4.11.17	[*iso*-phthaloyl chloride] 0.3125M (4mL)
	ME 4.11.18	
	ME 4.11.19	[*iso*-phthaloyl chloride] 0.4167M (3mL)
	ME 4.11.20	[*iso*-phthaloyl chloride] 0.625M (2mL)
		[*iso*-phthaloyl chloride] 1.25M (1mL)
stoichiometry		*iso*-phthaloyl chloride] 0.25M (5mL)
		[sodium hydroxide] 1.6M
	ME 4.11.21	[ornithine.HCl] 0.2M (5mL)
	ME 4.11.22	[ornithine.HCl] 0.3M (5mL)
	ME 4.11.23	[ornithine.HCl] 0.4M (5mL)
	ME 4.11.24	[ornithine.HCl] 0.5M (5mL)
	ME 4.11.25	[ornithine.HCl] 0.6M (5mL)
		[*iso*-phthaloyl chloride] 0.5M (5mL)
	ME 4.11.26	[ornithine.HCl] 0.2M (5mL)
		[sodium hydroxide] 1.6M
organic solvent	ME 4.11.27	hexane
	ME 4.11.28	chloroform
	ME 4.11.29	toluene
added salt	ME 4.11.30	1g
	ME 4.11.31	2g
	ME 4.11.32	3g
concentration of acid acceptor	ME 4.11.33	[sodium hydroxide] 2.0M
	ME 4.11.34	[sodium hydroxide] 1.8M
	ME 4.11.35	[sodium hydroxide] 1.6M
	ME 4.11.36	[sodium hydroxide] 1.4M
	ME 4.11.37	[sodium hydroxide] 1.2M
	ME 4.11.38	[sodium hydroxide] 1.0M
	ME 4.11.39	[sodium hydroxide] 0.3M

Table A11. Continued.

Experimental variable	Sample code	Experimental conditions
concentration of acid acceptor	ME 4.11.40	[sodium hydroxide] 0.2M
nature of acid acceptor	ME 4.11.41	[sodium carbonate] 0.8M
	ME 4.11.42	[triethylamine] 1.6M

Table A12. Summary of interfacial polycondensation reactions of *iso*-phthaloyl chloride with ornithine.HCl to investigate the solubilisation of organic solvent.

Experimental variable	Sample code	Experimental conditions	Yield
concentration of acid acceptor		*iso*-phthaloyl chloride] 0.25M (5mL)	
		[ornithine.HCl] 0.2M (5mL)	
	ME 4.12.1	[sodium hydroxide] 1.8M	92.2%
	ME 4.12.2	[sodium hydroxide] 1.6M	126%
	ME 4.12.3	[sodium hydroxide] 1.6M with 2.0M added at end of reaction	116%
	ME 4.12.4	[sodium hydroxide] 1.4M	N/A
	ME 4.12.5	[sodium hydroxide] 1.4M with 2.0M added at end of reaction	107%

Table A13. Summary of interfacial polycondensation of *iso*-phthaloy chloride with ornithine.HCl for determination of effect of LiBr concentration on the apparent molecular weight distribution determined by GPC.

Experimental variable	Sample code	Experimental conditions
homogenised for 10 minutes at speed 10 with no temperature control		[*iso*-phthaloyl chloride] 0.25M (5mL)
		[ornithine.HCl] 0.2M (5mL)
		[sodium hydroxide] 0.8M
	ME 4.13.1	fine head/fine generator
	ME 4.13.2	three blade impeller

Table A14. Summary of interfacial polycondensation reactions of diethylmalonyl chloride with selected diamines.

Diamines	Sample code	Experimental conditions	Yield
lysine ethyl ester.2HCl homogenised at speed 1 for30 minutes organic solvent CH_2Cl_2	ME 4.14.1	[diethylmalonyl chloride] 0.2M (10mL) [lysine ethyl ester.2HCl] 0.2M (10mL) [sodium hydroxide] 1.6M	0%
organic solvent Hexane	ME 4.14.2		0%
lysine ethyl ester.2HCl homogenised at speed 1 for30 minutes organic solvent CCl_4	ME 4.15.1	[diethylmalonyl chloride] 0.2M (10mL) [lysine ethyl ester.2HCl] 0.2M (10mL) [sodium carbonate] 1.6M	74%
	ME 4.15.2	[diethylmalonyl chloride] 0.2M (15mL) [lysine ethyl ester.2HCl] 0.2M (15mL) [sodium carbonate] 1.6M	84%
overhead stirrer at 2000r.p.m. for 30 minutes	ME 4.15.3.1	diethylmalonyl chloride] 0.2M (50mL) [lysine ethyl ester.2HCl] 0.2M (50mL) [potassium carbonate] 0.7M	80%
	ME 4.15.3.2	(hydrolysed sample)	
	ME 4.15.4	[diethylmalonyl chloride] 0.2M (300mL) [lysine ethyl ester.2HCl] 0.21M (300mL) [potassium carbonate] 0.7M	53.5%
hexamethylene diamine.2HCl homogenised at speed 1 for30 minutes	ME 4.16	[diethylmalonyl chloride] 0.2M (10mL) [hexamethylene diamine.2HCl] 0.2M (10mL) [sodium hydroxide] 1.6M	82%
lysine (free base) homogenised at speed 1 for 1 hr	ME 4.17.1	[diethylmalonyl chloride] 0.2M (10mL) [lysine free base] 0.2M (10mL) [sodium hydroxide] 1.6M	0%

Table A14. Continued.

Diamines	Sample code	Experimental conditions	Yield
lysine (free base) no organic solvent ratio diacyl chloride: diamine 2:1 homogenised at speed 1 for 1 hr	ME 4.17.1	0.3941g (0.004moles) diethylmalonyl chloride [lysine free base] 0.2M (10mL) [sodium hydroxide] 1.6M	0%
lysine ethyl ester.2HCl with approximately 10% w/w lysine (free base) homogenised at speed 1 for 1 hr	ME 4.18	[diethylmalonyl chloride] 0.2M (15mL) [lysine ethyl ester.2HCl] 0.2M (15mL) lysine (free base) 0.15g [sodium carbonate] 1.6M	46%
lysine ethyl ester.2HCl homogenised at speed 1 for 10 minutes then replace aqueous phase with lysine (free base) homogenised at speed 1 for 10 minutes	ME 4.19	[diethylmalonyl chloride] 0.2M (15mL) [lysine ethyl ester.2HCl] 0.2M (15mL) [sodium carbonate] 1.6M [lysine (free base)] 0.2M (15mL) [sodium carbonate] 1.6M	23%
lysine (free base) organic solvent $CHCl_3$ homogenised at speed 1 for 10 minutes	ME 4.20.1 ME 4.20.2	[diethylmalonyl chloride] 0.2M (10mL) [lysine (free base)] 0.2M (10mL) [sodium hydroxide] 1.6M [lysine (free base)] 0.4M (5mL) [sodium carbonate] 1.6M	0% 9%
lysine (free base) organic solvent CH_2Cl_2 homogenised at speed 1 for 10 minutes	ME 4.21.1 ME 4.21.2 ME 4.21.3	diethylmalonyl chloride] 0.2M (10mL) [lysine (free base)] 0.2M (10mL) [sodium carbonate] 1.6M [sodium carbonate] 1.2M [sodium carbonate] 0.8M	0% 0% 5%

Table A15. Summary of interfacial polycondensation reactions of diethylmalonyl chloride with diamines using accelerators.

Experimental Variables	Sample code	Experimental conditions	Yield
lysine ethyl ester.2HCl organic solvent CCl_4 homogenised at speed 1 for 10 minutes	ME 4.22	[diethylmalonyl chloride] 0.2M (15mL) [lysine ethyl ester.2HCl] 0.2M (15mL) [sodium carbonate] 1.6M sodium laurate 1% w/w	81%
lysine (Free Base) organic Solvent CCl_4 homogenised at speed 1 for 10 minutes	ME 4.23	[diethylmalonyl chloride] 0.2M (10mL) [lysine ethyl ester.2HCl] 0.2M (10mL) [sodium hydroxide] 1.6M sodium laurate 1% w/w	0%
lysine (free base) organic Solvent CH_2Cl_2 homogenised at speed 1 for 10 minutes	ME 4.24.1 ME 4.24.2	[diethylmalonyl chloride] 0.2M (10mL) [lysine ethyl ester.2HCl] 0.2M (10mL) [sodium carbonate] 1.6M benzalkonium bromide1% w/w benzalkonium bromide 0.2M	0% 0%
lysine (free base) organic solvent CH_2Cl_2 overhead stirrer at 2000 r.p.m. for 10 minutes	ME 4.25.1 ME 4.25.2 ME 4.25.3	[diethylmalonyl chloride] 0.2M (100mL) [lysine ethyl ester.2HCl] 0.2M (100mL) [potassium hydroxide] 1.6M 18 crown 6 0.02moles	0% (initially) (overnight precipitate) (precipitate on acidification after 24hrs)
lysine (free base) organic solvent CH_2Cl_2 homogenised at speed 1 for 10 minutes	ME 4.26.1 ME 4.26.2 ME 4.26.3	[diethylmalonyl chloride] 0.2M (100mL) [lysine ethyl ester.2HCl] 0.2M (100mL) [potassium hydroxide] 1.6M PEG 1000 0.02 moles PEG 2000 0.02 moles PEG 3000 0.02 moles	0% 0% 0%

Table A16. Summary of interfacial polycondensation reactions of dodecanedioyl chloride with diamines.

Diamines	Sample code	Experimental conditions	Yield
lysine (free base) organic solvent CCl_4 homogenised for 10 minutes at 0 °C	ME 4.27	[dodecane dioyl chloride] 0.25M (5mL) [lysine free base] 0.2M (5mL) [sodium hydroxide] 1.6M	crude precipitate 130% methanol washed precipitate 95%
lysine (free base) organic solvent hexane homogenised for 10 minutes at 0 °C	ME 4.28	[dodecane dioyl chloride] 0.25M (5mL) [lysine free base] 0.2M (5mL) [sodium hydroxide] 1.6M	crude precipitate 130%
ornithine.HCl organic solvent CCl_4 homogenised for 10 minutes at 0 °C	ME 4.29	dodecane dioyl chloride] 0.25M (5mL) [ornithine.HCl] 0.2M (5mL) [sodium hydroxide] 1.8M	crude precipitate 113%
ornithine.HCl organic solvent Hexane homogenised for 10 minutes at 0 °C	ME 4.30	[dodecane dioyl chloride] 0.25M (5mL) [ornithine.HCl] 0.2M (5mL) [sodium hydroxide] 1.8M	crude precipitate 130%

Table A17. Summary of interfacial polycondensation reactions of phenylmalonyl chloride with selected diamines.

Diamines	Sample code	Experimental conditions	Yield
lysine ethyl ester.2HCl organic solvent CCl_4 homogenised for 10 minutes at 0 °C	ME 4.31	[phenylmalonyl chloride] 0.2M (10mL) [lysine ethyl ester.2HCl] 0.2M (10mL) [sodium carbonate] 1.6M	40%
lysine ethyl ester.2HCl homogenised for 30 minutes at 0 °C	ME 4.32.1 ME 4.32.2	[phenylmalonyl chloride] 0.2M (10mL) [lysine ethyl ester.2HCl] 0.2M (10mL) [sodium carbonate] 1.6M (hydrolysed sample)	45%
lysine ethyl ester.2HCl with 1% sodium laurate homogenised for 10 minutes at 0 °C	ME 4.33	phenylmalonyl chloride] 0.2M (15mL) [lysine ethyl ester.2HCl] 0.2M (15mL) [sodium carbonate] 1.6M sodium laurate 1% w/w	47%
hexamethylene diamine.2HCl Homogenised for 10 minutes at 0 °C	ME 4.34	[phenylmalonyl chloride] 0.1M (10mL) [hexamethylene diamine.2HCl] 0.2M (10mL) [sodium hydroxide] 1.6M	65%
ornithine.HCl homogenised for 10 minutes at 0 °C	ME 4.35	[phenylmalonyl chloride] 0.2M (10mL) [ornithine.HCl] 0.2M (10mL) [sodium hydroxide] 1.6M	<5%

Table A18. Summary of interfacial polycondensation reactions of phenylglutaryl chloride with selected diamines.

Diamines	Sample code	Experimental conditions	Yield
lysine ethyl ester.2HCl organic solvent $CHCl_3$ overhead stirrer for 30 minutes	ME 4.36.1 ME 4.36.2	[phenylglutaryl chloride] 0.1M (300mL) [lysine ethyl ester.2HCl] 0.1M (300mL) [sodium carbonate] 0.4M (hydrolysed sample)	48%
lysine.HCl organic solvent $CHCl_3$ overhead stirrer for 10 minutes	ME 4.37	[phenylglutaryl chloride] 0.1M (30mL) [lysine.HCl] 0.1M (30mL) [sodium carbonate] 0.4M	precipitate on acidification 32%
lysine.HCl organic solvent CCl_4 overhead stirrer for 15 minutes	ME 4.38	phenylglutaryl chloride] 0.1M (30mL) [lysine.HCl] 0.1M (30mL) [sodium hydroxide] 1.6 M	precipitate on acidification 19.8%
lysine.HCl organic solvent Benzene overhead stirrer for 10 minutes	ME 4.39	phenylglutaryl chloride] 0.2M (20mL) [lysine.HCl] 0.2M 20mL) [sodium hydroxide] 1.6 M	precipitate on acidification 14.6%
ornithine.HCl organic solvent CCl_4 overhead stirrer for 10 minutes	ME 4.40	phenylglutaryl chloride] 0.1M (20mL) [ornithine.HCl] 0.1M (20mL) [sodium carbonate] 0.5M	precipitate on acidification 7%

Table A19. Summary of interfacial polycondensation reactions of ethylmalonyl chloride with selected diamines.

Diamines	Sample code	Experimental conditions	Yield
lysine ethyl ester.2HCl organic solvent CCl_4 homogenised at 0°C for 15 minutes	ME 4.41.1	[ethylmalonyl chloride] 0.2M (10mL) [lysine ethyl ester.2HCl] 0.2M (10mL) [sodium carbonate] 1.6M	69%
	ME 4.41.2	(hydrolysed sample)	

11. APPENDIX B

FT-IR SPECTRA AND BAND ASSIGNMENTS

Sample	FT-IR absorptions (cm^{-1})				
	3500-3000	2999-2000	1999-1500	1499-1300	1299-1000
ME 3.2.2	3480(sh) Free N-H stretch 3407(s) bonded N-H stretch 3071(s) bonded N-H stretch	2934(s) CH_2 C-H In Phase stretch 2867(s) CH_2 C-H Out of phase stretch	1735(s) C=O Ester stretch 1642(s)amide band I 1575(w) aryl skeletal ring breathing 1535(s) amide band II	1474(m) and 1443(m) CH_2 and CH_3 C-H asym. def. 1375(m) CH_3 sym. def. and COO^- sym. stretch	1296(m), 1209(s) and 1185(s) C-O stretch 1140(m) and 1091(m) aryl C-H in plane def. 1026(m) C-N stretch aliphatic amine and aryl C-H in plane def.
ME 3.2.8	3480(sh) Free N-H stretch 3413(s) bonded N-H stretch 3069(s) bonded N-H stretch	2936(s) CH_2 C-H In Phase stretch 2867(s) CH_2 C-H Out of phase stretch	1735(s) C=O Ester stretch 1642(s)amide band I 1582(w) aryl skeletal ring breathing 1535(s) amide band II	1478(m) and 1443(m) CH_2 and CH_3 C-H asym. def. 1371(m) CH_3 sym. def. and COO^- sym. stretch	1299(m), 1211(s) and 1185(s) C-O stretch 1140(m) and 1091(m) aryl C-H in plane def. 1023(m) C-N stretch aliphatic amine and aryl C-H in plane def.
ME 3.4.3	3382(s, broad) 3069(s) bonded N-H stretch	2936(s) CH_2 C-H In Phase stretch 2867(s) CH_2 C-H Out of phase stretch	1736(s) C=O Ester stretch 1637(s)amide band I 1582(w) aryl skeletal ring breathing 1535(s) amide band II	1474(m) and 1442(m) CH_2 and CH_3 C-H asym. def. 1373(m) CH_3 sym. def. and COO^- sym. stretch	1298(m), 1207(s) and 1185(s) C-O stretch 1139(m) and 1091(m) aryl C-H in plane def. 1026(m) C-N stretch aliphatic amine and aryl C-H in plane def.

Table B1. FT-IR band assignments for poly (lysine ethyl ester *iso*-phthalamide) synthesised using the miscible mixed solvent technique.

Sample	FT-IR absorptions (cm^{-1})				
	3500-3000	2999-2000	1999-1500	1499-1300	1299-1000
ME 3.5	3480(sh) Free N-H stretch 3407(s) bonded N-H stretch 3070(s) bonded N-H stretch	2934(s) CH_2 C-H In Phase stretch 2863(s) CH_2 C-H Out of phase stretch	1736(s) C=O Ester stretch 1642(s)amide band I 1575(sh) aryl skeletal ring breathing 1535(s) amide band II	1476(w) and 1443(w) CH_2 and CH_3 C-H asym. def. 1369(m) CH_3 sym. def. and COO^- sym. stretch	1298(w), 1209(s) and 1185(s) C-O stretch 1140(sh) and 1090(m) aryl C-H in plane def. 1024(m) C-N stretch aliphatic amine and aryl C-H in plane def.
ME 3.6.1	3480(sh) Free N-H stretch 3314(s) bonded N-H stretch 3068(s) bonded N-H stretch	2932(s) CH_2 C-H In Phase stretch 2861(s) CH_2 C-H Out of phase stretch	1737(s) C=O Ester stretch 1637(s)amide band I 1582(w) aryl skeletal ring breathing 1535(s) amide band II	1484(w) and 1443(w) CH_2 and CH_3 C-H asym. def. 1371(w) CH_3 sym. def. and COO^- sym. stretch	1298(w), 1209(s) and 1185(s) C-O stretch 1140(m) and 1091(m) aryl C-H in plane def. 1026(m) C-N stretch aliphatic amine and aryl C-H in plane def.
ME 3.6.2	3382(s, broad) 3064(s) bonded N-H stretch	2945(s) CH3 and CH_2 C-H In Phase stretch 2868(s) CH_2 C-H Out of phase stretch	1737(s) C=O Ester stretch 1637(s)amide band I 1581(w) aryl skeletal ring breathing 1535(s) amide band II	1476(m) and 1440(m) CH_2 and CH_3 C-H asym. def. 1369(m) CH_3 sym. def. and COO^- sym. stretch	1298(w), 1207(s) and 1179(s) C-O stretch 1138(m) and 1096(m) aryl C-H in plane def. 1026(m) C-N stretch aliphatic amine and aryl C-H in plane def.

Table B1. Continued.

Sample	FT-IR absorptions (cm^{-1})				
	3500-3000	2999-2000	1999-1500	1499-1300	1299-1000
ME 3.7.4.1	3480(sh) Free N-H stretch 3314(s) bonded N-H stretch 3068(s) bonded N-H stretch	2932(s) CH_2 C-H In Phase stretch 2861(s) CH_2 C-H Out of phase stretch	1790(m) anhydride sym. stretch 1730(s) C=O Ester stretch and anhydride asym. stretch 1637(s)amide band I 1582(w) aryl skeletal ring breathing 1535(s) amide band II	1474(w) and 1443(w) CH_2 and CH_3 C-H asym. def. 1367(m) CH_3 C-H sym. def. and COO^- sym. stretch	1296(s), 1211(s) and 1185(sh) C-O ester stretch 1140(m) and 1091(m) aryl C-H in plane def. 1068(m) C-O-C stretch 1024(m) C-N stretch aliphatic amine and aryl C-H in plane def.
ME 3.7.4.2	3314(s) bonded N-H stretch 3068(s) bonded N-H stretch	2945(s) CH3 and CH_2 C-H In Phase stretch 2861(s) CH_2 C-H Out of phase stretch	1734(s) C=O Ester stretch 1637(s)amide band I 1581(w) aryl skeletal ring breathing 1535(s) amide band II	1476(m) and 1440(m) CH_2 and CH_3 C-H asym. def. 1368(m)) CH_3 C-H sym. def. and COO^- sym. stretch	1298(s), 1207(s) and 1179(s) C-O ester stretch 1138(m) and 1096(m) aryl C-H in plane def. 1026(m) C-N stretch aliphatic amine and aryl C-H in plane def.
ME 3.8.3	3480(sh) Free N-H stretch 3407(s) bonded N-H stretch 3070(s) bonded N-H stretch	2934(s) CH_2 C-H In Phase stretch 2863(s) CH_2 C-H Out of phase stretch	1790(m) C=O anhydride sym. stretch 1736(sh) C=O Ester/anhydride asym. stretch 1695 C=O aryl acid 1637(s)amide band I 1576(sh) aryl skeletal ring breathing 1535(s) amide band II	1476(w) and 1443(w) CH_2 and CH_3 C-H asym. def. 1368(m)) CH_3 C-H sym. def. and COO^- sym. stretch	1298(m),1197(s) and 1179(s) C-O ester stretch 1253(w) amide III 1140(sh) and 1090(m) aryl C-H in plane def. 1071(m) C-O-C stretch 1039(m) C-N stretch aliphatic amine and aryl C-H in plane def.

Table B2. FT-IR band assignments for poly (lysine ethyl ester *iso*-phthalamide) and poly (lysine methyl ester *iso*-phthalamide) synthesised using the miscible mixed solvent technique with slow addition of the organic phase.

Sample	FT-IR absorptions (cm^{-1})				
	3500-3000	2999-2000	1999-1500	1499-1300	1299-1000
ME 3.9	3480(sh) Free N-H stretch 3407(s) bonded N-H stretch 3064(s) bonded N-H stretch	2934(s) CH_2 C-H In Phase stretch 2864(s) CH_2 C-H Out of phase stretch 2622(broad) bonded OH stretch	1707(s) C=O acid stretch 1636(s) amide band I 1575(w) aryl skeletal ring breathing 1535(s) amide band II	1475(m) and 1440(m) CH_2 and CH_3 C-H asym. def. 1357(w) COO^- sym. stretch 1300(s) coupled in plane C-O and OH def.	1179(m) COOH 1023(m) C-N stretch aliphatic amine and aryl C-H in plane def.
ME 3.10	3480(sh) Free N-H stretch 3270(s) bonded N-H stretch 3413(s) bonded N-H stretch 3064(s) bonded N-H stretch	2951(w) CH3 and CH_2 C-H In Phase stretch 2928(s) CH_2 C-H In Phase stretch 2854(w) CH_2 C-H Out of phase stretch	1701(s) C=O acid stretch 1636(s) amide band I 1535(s) amide band II	1476(m) and 1446(m) CH_2 and CH_3 C-H asym. def. 1425(m) aryl ring breathing 1357(w) COO^- sym. stretch	1298(s) coupled in plane C-O and OH def. 1215(sh) C-O stretch 1182(m) COOH 1145(sh) and 1091(w) aryl C-H in plane def. 1037(m) C-N stretch aliphatic amine and aryl C-H in plane def.
ME 3.11	3480(sh) Free N-H stretch 3324(s) bonded N-H stretch 3069(m) bonded N-H stretch	2934(s) CH_2 C-H In Phase stretch 2854(s) CH_2 C-H Out of phase stretch	1637(s) amide band I 1538(s) amide band II	1467(m) CH_2 C-H 1431(m) aryl ring breathing 1357(w) COO^- sym. stretch	1282(s) coupled in plane C-O and OH def. 1175(m) COOH 1140(w), 1071(m) aryl C-H in plane def. 1040(m) C-N stretch aliphatic amine and aryl C-H in plane def.

Table B3. FT-IR band assignments for poly (*iso*-phthalamides based on non esterified diamines using the miscible mixed solvent technique with slow addition of the organic phase.

Sample	FT-IR absorptions (cm^{-1})				
	3500-3000	2999-2000	1999-1500	1499-1300	1299-1000
ME 3.12.1	3480(sh) Free N-H stretch 3407(s) bonded N-H stretch 3070(s) bonded N-H stretch	2975(sh) CHECK 2934(s) CH_2 C-H In Phase stretch 2863(s) CH_2 C-H Out of phase stretch	1737(s) Ester stretch 1707(sh) acid stretch 1636(s)amide band I 1575(sh) aryl skeletal ring breathing 1541(s) amide band II	1476(w) and 1443(w) CH_2 and CH_3 C-H asym. def. 1369(m) CH_3 sym. def. and COO^- sym. stretch	1244 amide III 1215(s) and 1185(s) coupled CO and OH in plane def. and ester stretch 1140(sh) and 1090(m) aryl C-H in plane def. 1024(m) C-N stretch aliphatic amine and aryl C-H in plane def.
ME 3.12.2	3480(sh) Free N-H stretch 3369(s) bonded N-H stretch 3070(s) bonded N-H stretch	2933(s) CH_2 C-H In Phase stretch 2861(s) CH_2 C-H Out of phase stretch	1722(s) C=O Ester and acid stretch 1637(s)amide band I 1572(w) aryl skeletal ring breathing 1533(s) amide band II	1474(w) and 1442(w) CH_2 and CH_3 C-H asym. def. 1364(sh) CH_3 sym. def. and COO^- sym. stretch	1256 amide III 1220(m) and 1185(w) coupled CO and OH in plane def. and ester stretch 1140(sh) and 1091(m) aryl C-H in plane def. 1022(m) C-N stretch aliphatic amine and aryl C-H in plane def.
ME 3.13	3473(m) Free N-H stretch 3373(s) bonded N-H stretch 3068(s) bonded N-H stretch	2932(s) CH_2 C-H In Phase stretch 2854(s) CH_2 C-H Out of phase stretch	1709(s) C=O acid stretch 1637(s)amide band I 1581(w) aryl skeletal ring breathing 1531(s) amide band II	1474(m) and 1440(m) CH_2 and CH_3 C-H asym. def. 1369(m) COO^- sym. stretch	1282(s) coupled in plane C-O and OH def. 1211(sh) C-O stretch 1175(m) COOH 1138(sh) and 1096(sh) aryl C-H in plane def. 1026(m) C-N stretch aliphatic amine and aryl C-H in plane def.

Table B4. FT-IR band assignments for poly (*iso*-phthalamides) based on lysine and an additional diamine.

Sample	FT-IR absorptions (cm^{-1})				
	3500-3000	2999-2000	1999-1500	1499-1300	1299-1000
ME 3.14.	3343(s) bonded N-H stretch 3070(s) bonded N-H stretch	2969(s) CH_3 asym. stretch 2933(s) CH_2 C-H In Phase stretch 2880(w) CH_3 sym. stretch	1735(s) C=O ester stretch 1662(s) amide band I 1616(sh) N-H def. NH_2 1529(s) amide band II	1445(s) CH_2 C-H def. and CH_3 asym. def. and coupled CO and OH in plane deformation 1374(m) CH_3 sym. def. and COO^- asym. stretch 1345(w)-CH- def. 1304(sh) coupled CO stretch and OH in plane deformation	1260(sh) amide band III 1196(w) C-O stretch 1179(m) COOH 1026(m) C-N stretch aliphatic amine
ME 3.16.1	3343(s) bonded N-H stretch 3070(w) bonded N-H stretch	2963(m) CH_3 asym. stretch 2933(m) CH_2 C-H In Phase stretch 2881(w) CH_3 sym. stretch	1724(s) C=O acid stretch 1654(s) amide band I 1620(sh) N-H def. NH_2 1526(s) amide band II	1455(s) CH_2 C-H def. and CH_3 asym. def. 1383(s) CH_3 sym. def. 1344(w)-CH- def. 1305(sh) coupled CO stretch and OH in plane deformation	1259(sh) amide band III 1221(m) C-O stretch 1179(m) (COOH hydrolysed acid chloride) 1025(m) C-N stretch aliphatic amine

Table B5. FT-IR band assignments for poly (lysine ethyl ester diethylmalonamide) and poly (lysine diethylmalonamide) prepared using the miscible mixed solvent technique.

Sample	FT-IR absorptions (cm^{-1})				
	3500-3000	2999-2000	1999-1500	1499-1300	1299-1000
ME 4.1.1.1	3283(s) bonded N-H stretch 3081(m) bonded N-H stretch	2938(s) CH_2 C-H In Phase stretch 2866(m) CH_2 C-H Out of phase stretch	1735(s) C=O stretch ester 1577(w) sulphonamide N-H def. and/or aryl skeletal breathing	1453(sh) Aryl ring breathing and CH_2 C-H def. 1421(s) coupled C=O stretch and OH in plane def. 1369(sh) CH_3 sym. def. 1324(s) O=S=O asym. stretch	1293(sh) and 1173(m) coupled C=O stretch 1121(w) and 1082(w) aryl C-H in plane def. 1146 O=S=O sym. stretch 1026(m) C-N stretch aliphatic amine
ME 4.1.1.2	3283(s) bonded N-H stretch 3081(m) bonded N-H stretch	2938(s) CH_2 C-H In Phase stretch 2866(m) CH_2 C-H Out of phase stretch	1735(s) C=O stretch ester 1623(m) N-H def. NH_2 1581(w) sulphonamide N-H def. and/or aryl skeletal breathing	1453(sh) Aryl ring breathing and CH_2 C-H def. 1421(s) Aryl ring breathing 1370(m) CH_3 sym. def. 1331(s) O=S=O asym. stretch	1225(w) C-O stretch 1175(m), 1121(w) and 1082(w) aryl C-H in plane def. 1146 O=S=O sym. stretch 1026(m) C-N stretch aliphatic amine
ME 3.17.1	3283(s) bonded N-H stretch 3081(m) bonded N-H stretch	2938(s) CH_2 C-H In Phase stretch 2866(m) CH_2 C-H Out of phase stretch	1735(s) C=O stretch ester 1623(m) N-H def. NH_2 1581(w) sulphonamide N-H def. and/or aryl skeletal breathing	1453(sh) Aryl ring breathing and CH_2 C-H def. 1421(s) Aryl ring breathing 1370(m) CH_3 sym. def. 1331(s) O=S=O asym. stretch	1225(w) C-O stretch 1175(m), 1121(w) and 1075(w) aryl C-H in plane def. 1146 O=S=O sym. stretch 1026(m) C-N stretch aliphatic amine

Table B6 FT-IR band assignments for poly (lysine ethyl ester 1,3-benzene sulphonamide).

Sample	FT-IR absorptions (cm^{-1})				
	3500-3000	2999-2000	1999-1500	1499-1300	1299-1000
ME 3.17.2	3283(s) bonded N-H stretch 3081(m) bonded N-H stretch	2938(s) CH_2 C-H In Phase stretch 2866(m) CH_2 C-H Out of phase stretch	1726(s) C=O acid stretch 1620 N-H def. NH_3^+ 1581(sh) sulphonamide N-H def. and/or aryl skeletal; rig breathing	1453(sh) Aryl ring breathing and CH_2 C-H def. 1422(s) couples C=O and OH def. 1329(s) O=S=O asym. stretch	1225(w) 1175(sh) and C-O stretch 1082(w) aryl C-H in plane def. 1146 O=S=O sym. stretch 1026(m) C-N stretch aliphatic amine
ME 4.1.2	3283(s) bonded N-H stretch 3081(m) bonded N-H stretch	2938(s) CH_2 C-H In Phase stretch 2866(m) CH_2 C-H Out of phase stretch	1726(s) C=O stretch ester 1620(sh) N-H def. NH_3^+ 1581(sh) sulphonamide N-H def. and/or aryl skeletal; rig breathing	1455(m) Aryl ring breathing and CH_2 C-H def. 1417(s) couples C=O and OH def. 1331(s) O=S=O asym. stretch	1225(w) and 1175(sh) C-O stretch 1082(w) aryl C-H in plane def. 1146 O=S=O sym. stretch 1026(w) C-N stretch aliphatic amine

Table B7. FT-IR band assignments for poly (lysine 1,3-benzene sulphonamide).

Sample	FT-IR absorptions (cm^{-1})				
	3500-3000	2999-2000	1999-1500	1499-1300	1299-1000
ME 4.2.1	3480(sh) Free N-H stretch 3324(s) bonded N-H stretch 3070(s) bonded N-H stretch	2975(sh) CHECK 2934(s) CH_2 C-H In Phase stretch 2861(s) CH_2 C-H Out of phase stretch	1709(s) C=O acid stretch 1636(s)amide band I 1575(w) aryl skeletal ring breathing 1535(s) amide band II	1476(w) CH_2 def. 1442(m) CH_3 C-H asym. def. and coupled CO and OH in plane def.	1296(m) and 1179(sh) coupled CO and OH in plane def. and ester stretch 1221(m) C-O stretch 1024(m) C-N stretch aliphatic amine and aryl C-H in plane def.
ME 4.2.3.1	3330(b) bonded N-H stretch 3069(s) bonded N-H stretch 3063(s) bonded N-H stretch	2933(s) CH_2 C-H In Phase stretch 2861(s) CH_2 C-H Out of phase stretch	1735(s) C=O Ester and acid stretch 1644(s)amide band I 1579(sh) aryl skeletal ring breathing 1540(s) amide band II	1448(s) CH_2 and CH_3 C-H asym. def. (overlapped) 1377(m) CH_3 sym. def. and COO^- sym. stretch	1294(m) and 1188(w) coupled CO and OH in plane def. 1207(m) C-O stretch 1138(sh) and 1091(m) aryl C-H in plane def. 1019(m) C-N stretch aliphatic amine and aryl C-H in plane def.
ME 4.2.3.2	3480(sh) Free N-H stretch 3309(s) bonded N-H stretch 3068(s) bonded N-H stretch	2932(s) CH_2 C-H In Phase stretch 2854(s) CH_2 C-H Out of phase stretch	1737(s) C=O ester stretch 1637(s)amide band I 1579(w) aryl skeletal ring breathing 1531(s) amide band II	1482(m) 1438(m) CH_2 and CH_3 C-H def. 1377(m) CH_3 sym. def. and COO^- sym. stretch	1294(m) and 1188(w) coupled CO and OH in plane def. 1207(m) C-O stretch 1138(sh) and 1090(m) aryl C-H in plane def. 1024(s) C-N stretch aliphatic amine and aryl C-H in plane def.

Table B8. FT-IR band assignments for interfacially synthesised poly (*iso*-phthalamides) using CCl_4 as the organic solvent.

Sample	FT-IR absorptions (cm^{-1})				
	3500-3000	2999-2000	1999-1500	1499-1300	1299-1000
ME 4.2.5.1	3480(sh) Free N-H stretch 3324(s) bonded N-H stretch 3070(s) bonded N-H stretch	2979(sh) CHECK 2927(s) CH_2 C-H In Phase stretch 2861(s) CH_2 C-H Out of phase stretch	1737(s) C=O ester stretch 1644(s)amide band I 1581(w) aryl skeletal ring breathing 1535(s) amide band II	1474(w) and 1435(w) CH_2 and CH_3 C-H asym. def. 1369(m) CH_3 sym. def. and COO^- sym. stretch	1296(m) and 1179(sh) coupled CO and OH in plane def. and ester stretch 1253(s) amide III 1214(sh) C-O stretch 1024(s) C-N stretch aliphatic amine and aryl C-H in plane def.
ME 4.2.5.2	3330(b) bonded N-H stretch 3369(s) bonded N-H stretch 3070(s) bonded N-H stretch	2979(sh) CHECK 2927(s) CH_2 C-H In Phase stretch 2861(s) CH_2 C-H Out of phase stretch	1737(s) C=O Ester stretch 1644(s)amide band I 1581(sh) aryl skeletal ring breathing 1535(s) amide band II	1474(w) and 1435(w) CH_2 and CH_3 C-H asym. def. 1377(m) CH_3 sym. def. and COO^- sym. stretch	1296(m) and 1179(sh) coupled CO and OH in plane def. and ester stretch 1253(s) amide III 1138(sh) and 1091(m) aryl C-H in plane def. 1019(s) C-N stretch aliphatic amine and aryl C-H in plane def.
ME 4.2.6	3330(b) bonded N-H stretch 3369(s) bonded N-H stretch 3070(s) bonded N-H stretch	2932(s) CH_2 C-H In Phase stretch 2861(s) CH_2 C-H Out of phase stretch	1737(s) C=O Ester 1644(s)amide band I 1581(sh) aryl skeletal ring breathing 1533(s) amide band II	1474(w) and 1435(w) CH_2 and CH_3 C-H asym. def. 1377(m) CH_3 sym. def. and COO^- sym. stretch	1296(m) and 1179(sh) coupled CO and OH in plane def. and ester stretch 1253(s) amide III 1214(sh) C-O stretch 1138(sh) and 1091(m) aryl C-H in plane def. 1019(s) C-N stretch aliphatic amine and aryl C-H in plane def.

Table B9. FT-IR band assignments for interfacially synthesised poly (*iso*-phthalamides using $CHCl_3$ as the organic solvent.

Sample	FT-IR absorptions (cm^{-1})				
	3500-3000	2999-2000	1999-1500	1499-1300	1299-1000
4.3.1	3480(sh) Free N-H stretch 3324(s) bonded N-H stretch 3070(s) bonded N-H stretch	2979(sh) CHECK 2927(s) CH_2 C-H In Phase stretch 2864(s) CH_2 C-H Out of phase stretch	1736(s) C=O ester stretch 1641(s)amide band I 1577(w) aryl skeletal ring breathing 1541(s) amide band II	1477(w) and 1436(w) CH_2 and CH_3 C-H asym. def. 1377(m) CH_3 sym. def. and COO^- sym. stretch	1296(m) and 1177(sh) coupled CO and OH in plane def. and ester stretch 1209(m) C-O stretch 1141(sh) and 1091(m) aryl C-H in plane def. 1022(s) C-N stretch aliphatic amine and aryl C-H in plane def.
4.3.2	3330(b) bonded N-H stretch 3369(s) bonded N-H stretch 3070(s) bonded N-H stretch	2979(sh) CHECK 2927(s) CH_2 C-H In Phase stretch 2861(s) CH_2 C-H Out of phase stretch	1695(s) C=O acid stretch 1641(s)amide band I 1577(sh) aryl skeletal ring breathing 1541(s) amide band II	1477(w) CH_2 def. 1435(m) CH_3 C-H asym. def. and coupled CO and OH in plane def. 1377(sh) CH_3 sym. def. and COO^- sym. stretch	1298(m) and 1179(w) coupled CO and OH in plane def. and ester stretch 1227(s) C-O stretch 1141(sh) and 1091(m) aryl C-H in plane def. 1022(w) C-N stretch aliphatic amine and aryl C-H in plane def.

Table B10. FT-IR band assignments for lysine ethyl ester co lysine interfacial polycondensates with *iso*-phthaloyl chloride.

Sample	FT-IR absorptions (cm^{-1})				
	3500-3000	2999-2000	1999-1500	1499-1300	1299-1000
ME 4.4.1	3473(s) Free N-H stretch 3309(s) bonded N-H stretch 3068(s) bonded N-H stretch	2932(s) CH_2 C-H In Phase stretch 2864(s) CH_2 C-H Out of phase stretch	1701(sh) C=O acid stretch 1637(s)amide band I 1609 N-H def. NH_2 1535(s) amide band II	1474(w) CH_2 def. 1436(w) coupled CO and OH in plane def. 1374(m) COO^- sym. stretch	1296(M) and 1177(sh) coupled CO and OH in plane def. 1260(m) amide III 1141(w) and 1091(w) aryl C-H in plane def. 1022(w) C-N stretch aliphatic amine and aryl C-H in plane def.
ME 4.4.2	3330(b) bonded N-H stretch 3369(w) bonded N-H stretch 3070(w) bonded N-H stretch	2932(s) CH_2 C-H In Phase stretch 2861(s) CH_2 C-H Out of phase stretch 2550(b) bonded OH stretch	1707(s) C=O acid stretch 1636(s)amide band I 1576(m) aryl skeletal ring breathing 1541(s) amide band II	1482(w) CH_2 def. 1438(m) coupled CO and OH in plane def.	1292 amide III 1215(sh),and 1169(w) coupled CO and OH in plane def. 1022(w) C-N stretch aliphatic amine and aryl C-H in plane def.
ME 4.7	3330(b) bonded N-H stretch 3369(w) bonded N-H stretch 3070(w) bonded N-H stretch	2932(s) CH_2 C-H In Phase stretch 2861(s) CH_2 C-H Out of phase stretch 2550(b) bonded OH stretch	1789 C=O sym. anhydride stretch 1718(s) C=O antisymmetric anhydride (and acid stretch) 1636(s)amide band I 1541(s) amide band II	1433(m) coupled CO and OH in plane def.	1299(s) and 1186(s) C-O stretch coupled with OH in plane vib 1083(s) C-O-C anhydride stretch 1244 (m) amide III 1029(w) C-N stretch aliphatic amine and aryl C-H in plane def.

Table B11. FT-IR band assignments for hexamethylene diamine co lysine interfacial polycondensates with *iso*-phthaloyl chloride.

Sample	FT-IR absorptions (cm^{-1})				
	3500-3000	2999-2000	1999-1500	1499-1300	1299-1000
ME 4.5	3473(s) Free N-H stretch 3309(s) bonded N-H stretch 3070(s) bonded N-H stretch	2933(s) CH_2 C-H In Phase stretch 2861(s) CH_2 C-H Out of phase stretch 2550(w) bonded OH stretch	1789 C=O sym. anhydride stretch 1722(s) C=O antisymmetric anhydride and acid stretch 1637(s)amide band I 1579(sh) aryl skeletal ring breathing 1540(s) amide band II	1474(w) CH_2 def. 1436(w) coupled CO and OH in plane def. 1377(w) COO^- sym. stretch	1296(m) and 1175(sh) coupled CO and OH in plane def. 1260(m) amide III 1141(w) and 1091(w) aryl C-H in plane def. 1079(w) C-O-C anhydride stretch 1024(w) C-N stretch aliphatic amine and aryl C-H in plane def.
ME 4.6.1	3330(b) bonded N-H stretch 3369(w) bonded N-H stretch 3070(w) bonded N-H stretch	2932(s) CH_2 C-H In Phase stretch 2861(s) CH_2 C-H Out of phase stretch 2550(w) bonded OH stretch	1793 C=O sym. anhydride stretch 1722(sh) C=O antisymmetric anhydride and acid stretch 1696 C=O acid stretch 1644(s)amide band I 1572(sh) aryl skeletal ring breathing 1540(s) amide band II	1474(w) CH_2 def. 1435(m) coupled CO and OH in plane def. 1377(w) COO^- sym. stretch	1292(s) coupled CO and OH in plane def. 1218(sh),and 1175(w) coupled CO and OH in plane def. 1077(w) C-O-C anhydride stretch 1022(w) C-N stretch aliphatic amine and aryl C-H in plane def.

Table B12. FT-IR band assignments for hexamethylene diamine co ornithine interfacial polycondensates with *iso*-phthaloyl chloride.

Sample	FT-IR absorptions (cm^{-1})				
	3500-3000	2999-2000	1999-1500	1499-1300	1299-1000
ME 4.6.2	3473(s) Free N-H stretch 3309(s) bonded N-H stretch 3070(s) bonded N-H stretch	2933(s) CH_2 C-H In Phase stretch 2861(s) CH_2 C-H Out of phase stretch 2550(w) bonded OH stretch	1722(s) C=O antisymmetric anhydride and acid stretch 1644(s)amide band I 1572(sh) aryl skeletal ring breathing 1540(s) amide band II	1474(w) CH_2 def. 1435(w) CH_3 asym. def.	1296(m), 1214(m)and 1175(m) coupled CO and OH in plane def. and ester C-O stretch 1260(m) amide III 1141(w) and 1097(w) aryl C-H in plane def.
ME 4.6.3	3369(w) bonded N-H stretch 3298(b) bonded N-H stretch 3070(w) bonded N-H stretch	2932(s) CH_2 C-H In Phase stretch 2861(s) CH_2 C-H Out of phase stretch 2555(w) bonded OH stretch	1722(s) C=O ester and acid stretch 1683(s) C=O aromatic carboxylic acid stretch 1631(s) amide band I 1572(sh) aryl skeletal ring breathing 1540(s) amide band II	1474(w) CH_2 def. 1435(s) CH_3 asym. def.	1296(s), 1214(m)and 1175(m) coupled CO and OH in plane def. and ester C-O stretch 1262(m) amide III 1145(w) and 1090(w) aryl C-H in plane def. 1022(w) C-N stretch aliphatic amine and aryl C-H in plane def.

Table B12. Continued.

Sample	FT-IR absorptions (cm^{-1})				
	3500-3000	2999-2000	1999-1500	1499-1300	1299-1000
ME 4.11.14	3480(sh) free N-H stretch 3373(b) bonded N-H stretch 3070(w) bonded N-H stretch	2932(m) CH_2 C-H In Phase stretch 2875(s) CH_2 C-H Out of phase stretch 2555(w) bonded OH stretch	1700 C=O acid stretch 1640 amide band I 1580(sh) aryl skeletal ring breathing 1539 amide band II	1481(w) CH_2 def. 1445(m) coupled CO and OH in plane def.	1298(m) coupled CO and OH in plane def. 1233(sh) C-O stretch 1142(sh) aryl C-H in plane def.
ME 4.11.25	3262(s) OH stretch 3063(w) bonded N-H stretch	2933(m) CH_2 C-H In Phase stretch 2881(w) 2816(w) and 2602(w) bonded OH stretch	1706 C=O acid stretch 1640 amide band I 1580(sh) aryl skeletal ring breathing 1540 amide band II	1481(w) aryl skeletal ring breathing and CH_2 def. 1454 and 1422(m) coupled CO and OH in plane def.	1299(m) coupled CO and OH in plane def. 1246(m) amide III 1220(sh) C-O stretch
ME 4.11.26	3413(sh) bonded OH stretch 3063(w) bonded N-H stretch 3006(w) bonded OH stretch	2666(m) and 2555(m) bonded OH stretch	1694(b,s) C=O aryl and aliphatic acid stretch 1642 amide band I 1611(s) COO^- asym. stretch 1580(sh) aryl skeletal ring breathing 1542 amide band II	1485 (w) aryl skeletal ring breathing and CH_2 def. 1422(s) coupled C=O and OH in plane def.	1292(s) and 1165(m) coupled CO and OH in plane def. 1225(sh) C-O stretch

Table B13. FT-IR band assignments for poly (ornithine *iso*-phthalamides) synthesised by the interfacial method.

Sample	FT-IR absorptions (cm^{-1})				
	3500-3000	2999-2000	1999-1500	1499-1300	1299-1000
ME 4.11.32	3480(sh) free N-H stretch 3373(b) bonded N-H stretch 3073(w) bonded N-H stretch	2934(m) CH_2 C-H In Phase stretch 2867(s) CH_2 C-H Out of phase stretch 2555(w) bonded OH stretch	1703 C=O acid stretch 1640 amide band I 1579(sh) aryl skeletal ring breathing 1542 amide band II	1476(w) CH_2 def. 1439(m) coupled CO and OH in plane def.	1296(s) coupled CO and OH in plane def. 1233(sh) C-O stretch 1142(sh) aryl C-H in plane def.
ME 4.11.33	3373(b) bonded N-H stretch 3073(w) bonded N-H stretch	2932(s) CH_2 C-H In Phase stretch 2861(s) CH_2 C-H Out of phase stretch 2555(w) bonded OH stretch	1701 C=O acid stretch 1640 amide band I 1579(sh) aryl skeletal ring breathing 1542 amide band II	1474(w) aryl skeletal ring breathing and CH_2 def. 1454 and 1422(m) coupled CO and OH in plane def.	1296(s) coupled CO and OH in plane def. 1233(sh) C-O stretch 1142(w) aryl C-H in plane def.
ME 4.11.42	3480(sh) free N-H stretch 3373(b) bonded N-H stretch 3067(w) bonded N-H stretch	2932(s) CH_2 C-H In Phase stretch 2861(s) CH_2 C-H Out of phase stretch 2555(w) bonded OH stretch	1704 C=O acid stretch 1640 amide band I 1579(sh) aryl skeletal ring breathing 1542 amide band II	1479(w) CH_2 def. 1443(m) coupled CO and OH in plane def.	1296(m) coupled CO and OH in plane def. 1233(sh) C-O stretch 1142(sh) aryl C-H in plane def.

Table B13. Continued.

Sample	FT-IR absorptions (cm^{-1})				
	3500-3000	2999-2000	1999-1500	1499-1300	1299-1000
ME 4.15.1	3343(s) bonded N-H stretch 3070(w) bonded N-H stretch	2969(m) CH_3 asym. stretch 2933(m) CH_2 C-H In Phase stretch 2875(w) CH_3 sym. stretch 2855 CH_2 C-H out of phase stretch	1735(s) C=O ester stretch 1654(s) amide band I 1620(sh) N-H def. NH_2 1526(s) amide band II	1455(m) CH_2 C-H def. and CH_3 asym. def. 1374(s) CH_3 sym. def. and COO^- asym. stretch 1345(w)-CH- def. 1300(sh) coupled CO and OH in plane def.	1259(w) amide band III 1201(m) C-O stretch ester 1175(m) COOH 1026(m) C-N stretch aliphatic amine
ME 4.15.3.1	3343(s) bonded N-H stretch 3070(w) bonded N-H stretch	2963(m) CH_3 asym. stretch 2933(m) CH_2 C-H In Phase stretch 2881(s) CH_3 sym. stretch	1737(s) C=O ester stretch 1657(s) amide band I 1620(sh) N-H def. NH_2 1526(s) amide band II	1455(s) CH_2 C-H def. and CH_3 asym. def. 1374(s) CH_3 sym. def. and COO^- asym. stretch 1344(w)-CH- def. 1300(w) coupled C-O and OH in plane def.	1259(w) amide band III 1201(m) C-O stretch ester 1175(m) COOH 1026(m) C-N stretch aliphatic amine
ME 4.15.4	3343(s) bonded N-H stretch 3070(w) bonded N-H stretch	2963(m) CH_3 asym. stretch 2933(m) CH_2 C-H In Phase stretch 2881(s) CH_3 sym. stretch	1737(s) C=O ester stretch 1657(s) amide band I 1620(sh) N-H def. NH_2 1526(s) amide band II	1455(s) CH_2 C-H def. and CH_3 asym. def. 1374(s) CH_3 sym. def. and COO^- asym. stretch 1344(w)-CH- def. 1300(w) coupled C-O and OH in plane def.	1259(w) amide band III 1201(m) C-O stretch ester 1175(m) COOH 1025(m) C-N stretch aliphatic amine

Table B14. FT-IR band assignments for poly (lysine ethyl ester diethylmalonamide).

Sample	FT-IR absorptions (cm^{-1})				
	3500-3000	2999-2000	1999-1500	1499-1300	1299-1000
ME 4.16	3343(s) bonded N-H stretch 3070(w) bonded N-H stretch	2961(m) CH_3 asym. stretch 2932(m) CH_2 C-H In Phase stretch 2875(w) CH_3 sym. stretch 2859(CH_2 C-H out of phase stretch	1654(s) amide band I 1614(sh) N-H def. NH_2 1527(s) amide band II	1448(m) CH_2 C-H def. and CH_3 asym. def. 1374(sh) CH_3 sym. def. and COO^- asym. stretch 1303(w) coupled C-O and OH in plane def.	1259(m) amide band III 1168(m) COOH (hydrolysed acid chloride) 1026(m) C-N stretch aliphatic amine
ME 4.18	3343(s) bonded N-H stretch 3070(w) bonded N-H stretch	2963(m) CH_3 asym. stretch 2933(m) CH_2 C-H In Phase stretch 2881(s) CH_3 sym. stretch	1741(s) C=O ester stretch 1663(s) amide band I 1620(sh) N-H def. NH_2 1526(s) amide band II	1455(s) CH_2 C-H def. and CH_3 asym. def. 1383(s) CH_3 sym. def. and COO^- asym. stretch 1344(w)-CH- def. 1300(w) coupled C-O and OH in plane def.	1259(w) amide band III 1220(m) and 1201(m) C-O stretch 1175(m) COOH 1026(m) C-N stretch aliphatic amine
ME 4.19	Broad bands with indistinguishable peaks	Broad bands with indistinguishable peaks	1722(s, broad) C=O ester and acid overlapped 1657(s) amide band I 1620(sh) N-H def. NH_2 1527(s) amide band II	1455(s) CH_2 C-H def. and CH_3 asym. def. 1383(s) CH_3 sym. def. 1344(w)-CH- def. 1300(w) coupled C-O and OH in plane def.	1220(m) coupled C-O and OH in plane def. 1175(m) COOH 1026(m) C-N stretch aliphatic amine

Table B15. FT-IR band assignments for poly (hexamethylene diethylmalonamide) and modified poly (lysine ethyl ester diethylmalonamides).

Sample	FT-IR absorptions (cm^{-1})				
	3500-3000	2999-2000	1999-1500	1499-1300	1299-1000
ME 4.15.3.2	3343(s) bonded N-H stretch 3070(w) bonded N-H stretch	2961(m) CH_3 asym. stretch 2932(m) CH_2 C-H In Phase stretch 2875(w) CH_3 sym. stretch	1707(s) C=O acid stretch 1654(s) amide band I 1618(sh) N-H def. NH_3^+ 1523(s) amide band II	1448(m) CH_2 C-H def. and CH_3 asym. def. 1381(sh) CH_3 sym. def. 1303(w)) coupled C-O and OH in plane def.	1262(m) amide band III 1173(m) COOH 1221(s) C-O stretch 1026(m) C-N stretch aliphatic amine

Table B16. FT-IR band assignments for hydrolysed poly (lysine ethyl ester diethylmalonamide).

Sample	FT-IR absorptions (cm^{-1})				
	3500-3000	2999-2000	1999-1500	1499-1300	1299-1000
ME 4.22	3343(s) bonded N-H stretch 3070(w) bonded N-H. stretch	2961(m) CH_3 asym. stretch 2932(m) CH_2 C-H In Phase stretch 2875(w) CH_3 sym. stretch	1741(s) C=O ester stretch 1663(s) amide band I 1572(s) COO^- asym. stretch (sodium laurate) 1523(s) amide band II	1455(m) CH_2 C-H def. and CH_3 asym. def. 1383(sh) CH_3 sym. def. and COO^- asym. stretch 1344(w) -CH- def. 1303(w) coupled C-O and OH in plane def.	1262(m) amide band III 1173(sh) COOH 1026(m) C-N stretch aliphatic amine
ME 4.23 Aqueous phase	3343(s) bonded N-H stretch	2966(m) CH_3 asym. stretch 2932(m) CH_2 C-H In Phase stretch 2875(w) CH_3 sym. stretch	1715(s) C=O acid stretch 1637(sh) amide band 1598 Overlapping NH_3^+ def. and COO^- asym. stretch 1533(m) amide band II	1447(s) CH_2 C-H def. and CH_3 asym. def. 1409(m) coupled CO and OH in plane def. 1377(m) CH_3 sym. def. and COO^- asym. stretch 1338(w) -CH- def.	1221(m) C-O stretch 1181(m) COOH 1030(w) aliphatic amine C-N stretch
ME 4.24 Aqueous phase		2966(m) CH_3 asym. Stretch 2932(m) CH_2 C-H In Phase stretch 2875(w) CH_3 sym. stretch 2855 CH_2 C-H out of phase stretch	1637(s) amide band 1579(s) COO^- asym. stretch 1533(w) amide band II 1447(s) CH_2 C-H def. and CH_3 asym. def. 1409(m) COO^- sym. stretch	1447(s) CH_2 C-H def. and CH_3 asym. def. and COO^- sym. stretch 1409(m) coupled CO and OH in plane def. 1364(m) CH_3 sym. def.-C-$(CH_3)_3$ and COO^- asym. stretch	1221(m) C-O stretch 1181(m) COOH 1030(w) aliphatic amine C-N stretch

Table B17. FT-IR band assignments for interfacial polycondensates of diethylmalonyl chloride and diamines in the presence of accelerators.

Sample	FT-IR absorptions (cm^{-1})				
	3500-3000	2999-2000	1999-1500	1499-1300	1299-1000
ME 4.25.1	3600(b) OH stretch	2966(s) CH_3 asym. stretch 2933(s) CH_2 C-H In Phase stretch 2875(s) CH_3 sym. stretch	1820(m) C=O sym. anhydride stretch 1765(sh) C=O asym. stretch anhydride 1717(s)C=O acid stretch	1663(m) amide band I 1608(m) N-H stretch NH_2 1530(m) amide band II 1455 CH_2 C-H def. and CH_3 asym. def. 1385 CH_3 sym. def. 1347 -CH- def.	1246 amide band III 1077 C-O-C stretch
ME 4.25.2	3414(s) bonded OH	2957(m) CH_3 asym. stretch 2932(sh) CH_2 C-H In Phase stretch 2875(sh) CH_3 sym. stretch 2625 (broad) bonded OH stretch	1719(s) C=O acid stretch	1452(m) CH_2 C-H def. and CH_3 asym. def. 1411 C-O stretch coupled with OH in plane vib	1298(sh) coupled C-O and OH in plane def. 1256(b) C-O stretch 1162(sh) COOH

Table B18. FT-IR band assignment for products from the interfacial polycondensation of lysine and diethylmalonyl chloride in the presence of 18-C-6.

Sample	FT-IR absorptions (cm^{-1})				
	3500-3000	2999-2000	1999-1500	1499-1300	1299-1000
ME4.27	3291(s) bonded N-H stretch 3076(s) bonded N-H stretch	2920(s) CH_2 C-H In Phase stretch 2848(s) CH_2 C-H Out of phase stretch	1696(s) C=O Acid stretch 1637(s) Amide band I 1540(s) Amide band II	1459(m) and 1430(m) CH_2 C-H def. 1404(w) C-O vib coupled with OH in plane def. 1377(m) COO^- sym. stretch	1278(w),coupled C-O vib and OH in plane def. 1225(m) and 1188(w) C-O stretch
ME4.28	3291(s) bonded N-H stretch 3076(s) bonded N-H stretch	2920(s) CH_2 C-H In Phase stretch 2848(s) CH_2 C-H Out of phase stretch	1701(s) C=O Acid stretch 1637(s) Amide band I 1540(s) Amide band II	1459(m) and 1434(m) CH_2 C-H def. 1404(w) C-O vib coupled with OH in plane def. 1377(m) COO^- sym. stretch	1278(w),coupled C-O vib and OH in plane def. 1225(m) and 1181(w) C-O stretch
ME4.29	3311(s) bonded N-H stretch 3076(s) bonded N-H stretch	2920(s) CH_2 C-H In Phase stretch 2848(s) CH_2 C-H Out of phase stretch	1696(s) C=O Acid stretch 1644(s) Amide band I 1540(s) Amide band II	1461(m) and 1430(m) CH_2 C-H def. 1404(w) C-O vib coupled with OH in plane def. 1331(m) COO^- sym. stretch	1279(w),coupled C-O vib and OH in plane def. 1220(m) and 1181(w) C-O stretch
ME4.30	3311(s) bonded N-H stretch 3076(s) bonded N-H stretch	2920(s) CH_2 C-H In Phase stretch 2848(s) CH_2 C-H Out of phase stretch	1701(s) C=O Acid stretch 1637(s) Amide band I 1540(s) Amide band II	1459(m) and 1434(m) CH_2 C-H def. 1404(w) C-O vib coupled with OH in plane def. 1331(m) COO^- sym. stretch	1279(w),coupled C-O vib and OH in plane def. 1225(m) and 1181(w) C-O stretch

Table B19. FT-IR band assignments for poly (lysine dodecamide) and poly (ornithine dodecamide).

Sample	FT-IR absorptions (cm^{-1})				
	3500-3000	2999-2000	1999-1500	1499-1300	1299-1000
ME 4.31	3296(s) bonded N-H stretch 3062(m) bonded N-H stretch 3029(w) aryl C-H stretch	2977(w) N-H stretch NH_3^+ 2931(s)) CH_2 C-H In Phase stretch 2860(m) CH_2 C-H Out of phase stretch	1737(s) C=O stretch ester 1668(s) Amide band I 1596(w) COO^- asym. stretch 1525(s) Amide band II	1492(m) Aryl ring breathing 1447(m) Aryl ring breathing and CH_2 C-H def. 1369(m) CH_3 sym. def. and COO^- asym. stretch 1303(w) coupled C-O vib and OH in plane def.	1258(sh) Amide band III 1199(s) C-O ester stretch 1025(w) C-N stretch aliphatic amine
ME 4.32.1	3296(s) bonded N-H stretch 3062(m) bonded N-H stretch 3029(w) aryl C-H stretch	2977(w) N-H stretch NH_3^+ 2931(s)) CH_2 C-H In Phase stretch 2860(m) CH_2 C-H Out of phase stretch	1737(s) C=O stretch ester 1668(s) Amide band I 1596(w) COO^- asym. stretch 1525(s) Amide band II	1492(m) Aryl ring breathing 1447(m) Aryl ring breathing and CH_2 C-H def. 1364(m) CH_3 sym. def. and COO^- asym. stretch 1303(w) coupled C-O and OH in plane def.	1258(sh) Amide band III 1199(s) C-O ester stretch 1025(w) C-N stretch aliphatic amine
ME 4.32.2	3296(s) bonded N-H stretch	2919(s)) CH_2 C-H In Phase stretch 2853(m) CH_2 C-H Out of phase stretch	1711(s) C=O acid ester 1668(s) Amide band I 1525(s) Amide band II	1492(m) Aryl ring breathing 1447(m) Aryl ring breathing and CH_2 C-H def. 1364(m) CH_3 sym. def. and COO^- asym. stretch 1303(w) coupled C-O and OH in plane def.	1258(sh) Amide band III 1199(s) C-O ester stretch 1025(w) C-N stretch aliphatic amine

Table B20. FT-IR band assignments for poly (lysine ethyl ester phenylmalonamides).

Sample	FT-IR absorptions (cm^{-1})				
	3500-3000	2999-2000	1999-1500	1499-1300	1299-1000
ME 4.33	3296(s) bonded N-H stretch 3062(m) bonded N-H stretch 3029(w) aryl C-H stretch	2977(w) N-H stretch NH_3^+ 2931(s)) CH_2 C-H In Phase stretch 2860(m) CH_2 C-H Out of phase stretch	1737(s) C=O stretch ester 1668(s) Amide band I 1596(w) COO^- asym. stretch 1525(s) Amide band II	1492(m) Aryl ring breathing 1443(m) Aryl ring breathing and CH_2 C-H def. 1377(m) CH_3 sym. def. and COO^- sym. stretch 1303(w) coupled C-O vib and OH in plane def.	1258(sh)Amide band III 1199(s) C-O ester stretch 1025(w) C-N stretch aliphatic amine
ME 4.34	3415(sh) bonded N-H stretch 3298(s) bonded N-H stretch 3063(m) bonded N-H stretch 3031(w) aryl C-H stretch	2927(s) CH_2 C-H In Phase stretch 2855(s) CH_2 C-H Out of phase stretch	1663(s) Amide band I 1598(w) COO^- asym. stretch 1533(s) Amide band II	1487(m) Aryl ring breathing 1448(m) Aryl ring breathing and CH_2 C-H def. 1377(m) CH_3 sym. def. and COO^- sym. stretch	1214(m) C-O acid stretch

Table B21. FT-IR band assignments for poly (lysine ethyl ester phenylmalonamide) synthesised interfacially with surfactant and poly (hexamethylene diamine phenylmalonamide).

Sample	FT-IR absorptions (cm^{-1})				
	3500-3000	2999-2000	1999-1500	1499-1300	1299-1000
ME 4.36.1	3291(s) bonded N-H stretch 3063(m) bonded N-H stretch 3031(w) Aryl C-H stretch	2933 (s) CH_2 C-H In Phase stretch 2861(s) CH_2 C-H Out of phase stretch	1735(s) C=O Ester stretch 1637(s) Amide band I 1616(w) NH_2 def 1546(s) Amide band II	1494(w) Aromatic ring breathing 1448(m) Aromatic ring breathing and CH_2 C-H def. 1370(m) CH_3 sym. def. and COO^- asym. stretch	1292(w), 1188(m) and 1155(w) C-O stretch 1259(w) Amide III 1097(w) and 1155(w) aryl C-H in plane def. 1023(s) C-N stretch aliphatic amine
ME 4.36.2	3467 N-H stretch free amine 3408(s) bonded N-H stretch 3291(sh) bonded N-H stretch 3063(m) bonded N-H stretch 3031(w) Aryl C-H stretch	2954(s) and 2933 (s) CH_2 C-H In Phase stretch 2861(s) CH_2 C-H Out of phase stretch	1735(s) C=O Ester stretch 1637(s)Amide band I 1546(s) Amide band II	1494(w) Aromatic ring breathing 1448(m) Aromatic ring breathing and CH_2 C-H def. 1370(m) CH_3 sym. def. and COO^- asym. stretch	1292(w), 1188(m) and 1155(w) C-O stretch 1259(w) Amide III 1097(w) and 1155(w) aryl C-H in plane def. 1023(s) C-N stretch aliphatic amine

Table B22. FT-IR band assignments for poly (lysine ethyl ester phenylglutamide).

Sample	FT-IR absorptions (cm^{-1})				
	3500-3000	2999-2000	1999-1500	1499-1300	1299-1000
ME 4.37	3467 N-H stretch free amine 3415(s) bonded N-H stretch 3252(sh) bonded N-H stretch 3070(m) bonded N-H stretch 3031(w) Aryl C-H stretch	2959(s) N-H stretch NH_3^+ 2927 (s) CH_2 C-H In Phase stretch 2861(s) CH_2 C-H Out of phase stretch	1715(s) C=O Acid stretch 1637(s) Amide band I 1611(s) N-H def. NH_3^+ 1546(s) Amide band II	1494(w) Aromatic ring breathing 1448(m) Aromatic ring breathing and CH_2 C-H def. 1403w) C-O vib coupled with OH in plane def. 1377(w) COO^- sym. stretch	1298(sh) coupled CO and OH def. 1227(s) and 1162(sh) C-O stretch 1025(w) C-N stretch aliphatic amine
ME 4.40	3467 N-H stretch free amine 3408(s) bonded N-H stretch 3252(w) bonded N-H stretch 3063(m) bonded N-H stretch 3031(w) Aryl C-H stretch	2933(s) CH_2 C-H In Phase stretch 2848(s) CH_2 C-H Out of phase stretch	1715(s) C=O Acid stretch 1636(s) Amide band I 1546(s) Amide band II	1494(w) Aromatic ring breathing 1448(m) Aromatic ring breathing and CH_2 C-H def. 1403w) C-O vib coupled with OH in plane def. 1377(w) COO^- sym. stretch	1298(sh) coupled CO and OH def. 1233(s) and 1162(sh) C-O stretch 1025(w) C-N stretch aliphatic amine

Table B23. FT-IR band assignments for poly (lysine phenylglutamide) and poly (ornithine phenylglutamide).

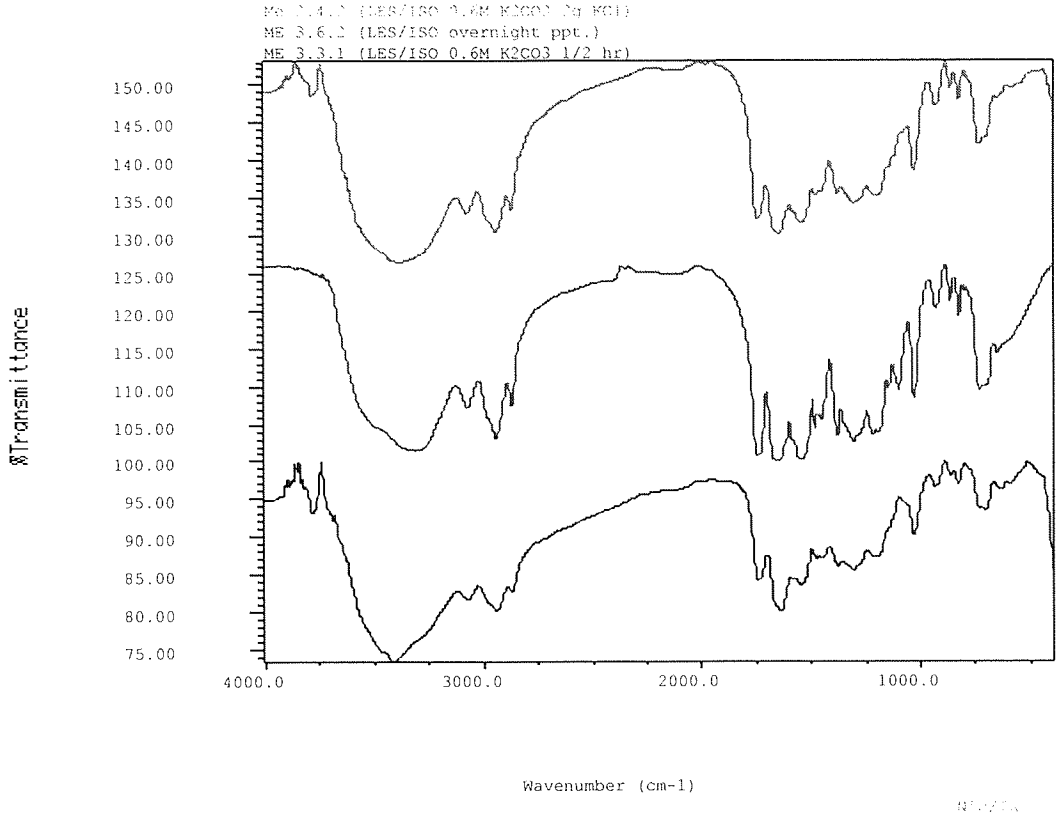
Sample	FT-IR absorptions (cm^{-1})				
	3500-3000	2999-2000	1999-1500	1499-1300	1299-1000
ME 4.8	3450(s) bonded N-H stretch 3057(w) $=CH_2$ C-H Stretch	2925(m) CH_2 C-H In Phase stretch 2859(m) CH_2 C-H Out of phase stretch	1734 C=O ester stretch 1648 Amide band I 1556(m) and 1543(m) Amide Band II	1457(m) CH_2 C-H def. 1442(m) CH_3 asym. def. 1377(m) CH_3 sym. def. and COO^- asym. def.	1292(w) and 1132(m) C-O ester stretch 1025(m) C-N stretch aliphatic amine

Table B24. FT-IR band assignments for poly (lysine ethyl ester itaconamide).

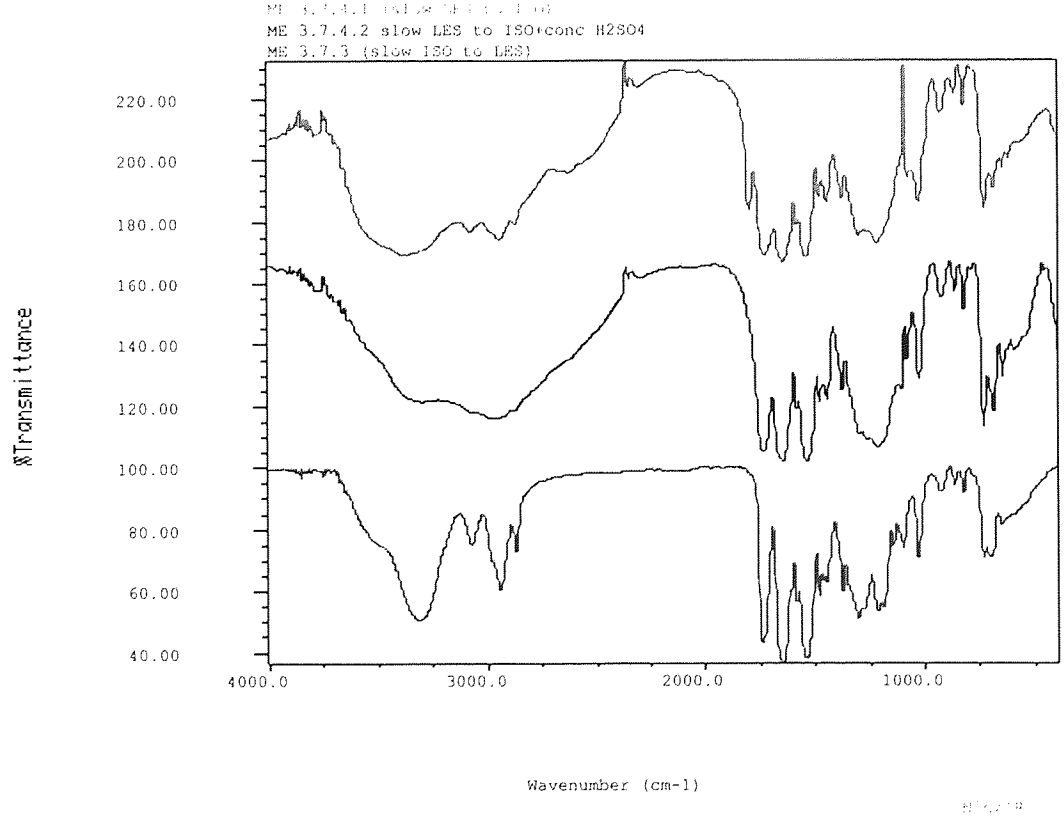
Sample	FT-IR absorptions (cm^{-1})				
	3500-3000	2999-2000	1999-1500	1499-1300	1299-1000
ME 4.41.1	3343(s) bonded N-H stretch 3070(w) bonded N-H stretch	2969(m) CH_3 asym. stretch 2933(m) CH_2 C-H In Phase stretch 2875(w) CH_3 sym. stretch 2855 CH_2 C-H out of phase stretch	1737(s) C=O ester stretch 1650(s) amide band I 1620(sh) N-H def. NH_2 1530(s) amide band II	1455(m) CH_2 C-H def. and CH_3 asym. def. 1374(s) CH_3 sym. def. and COO^- asym. stretch 1345(w)-CH- def.	1296(sh) C-O ester stretch 1264(w) amide band III 1200(m) C-O stretch ester 1175(m) COOH 1026(m) C-N stretch aliphatic amine

Table B25. FT-IR band assignments for poly (lysine ethyl ester ethylmalonamide).

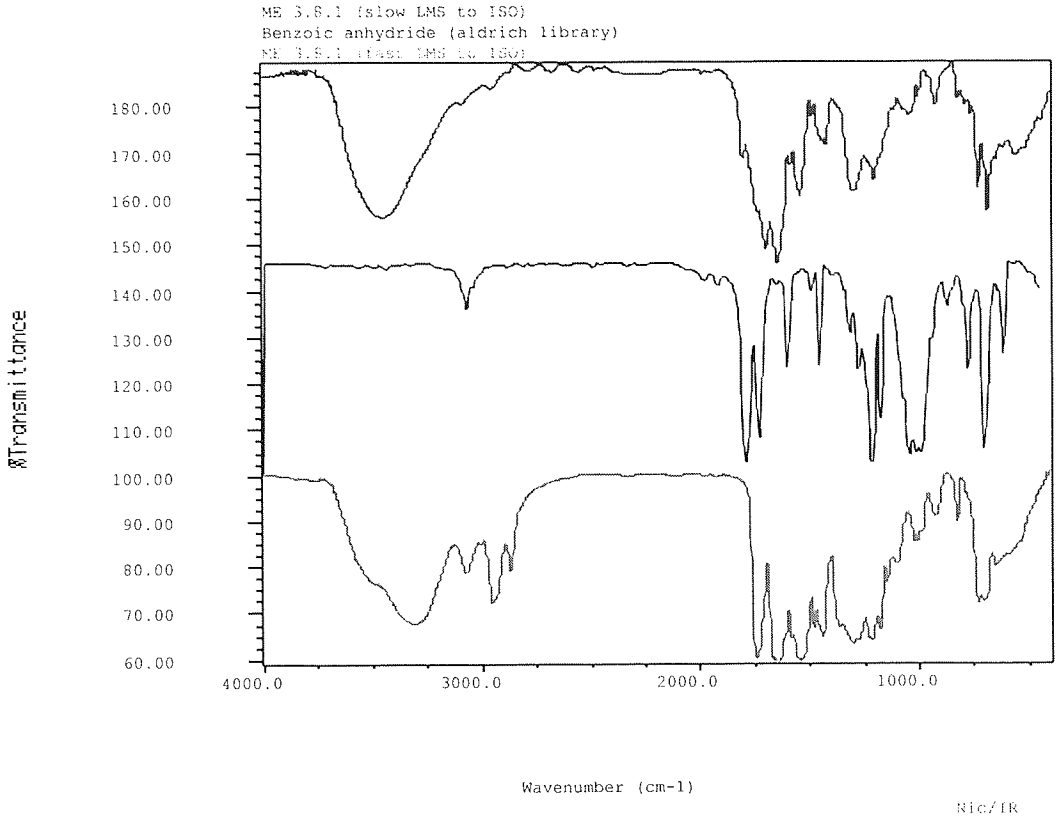
Spectrum 1.



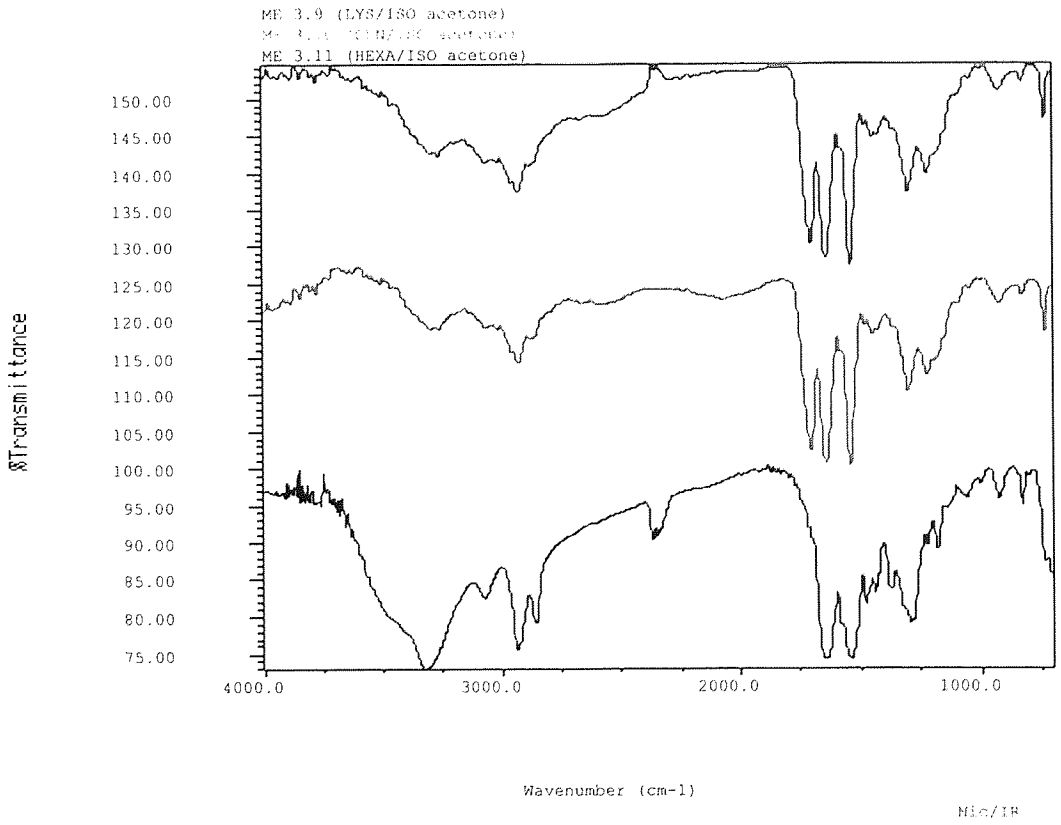
Spectrum 2.



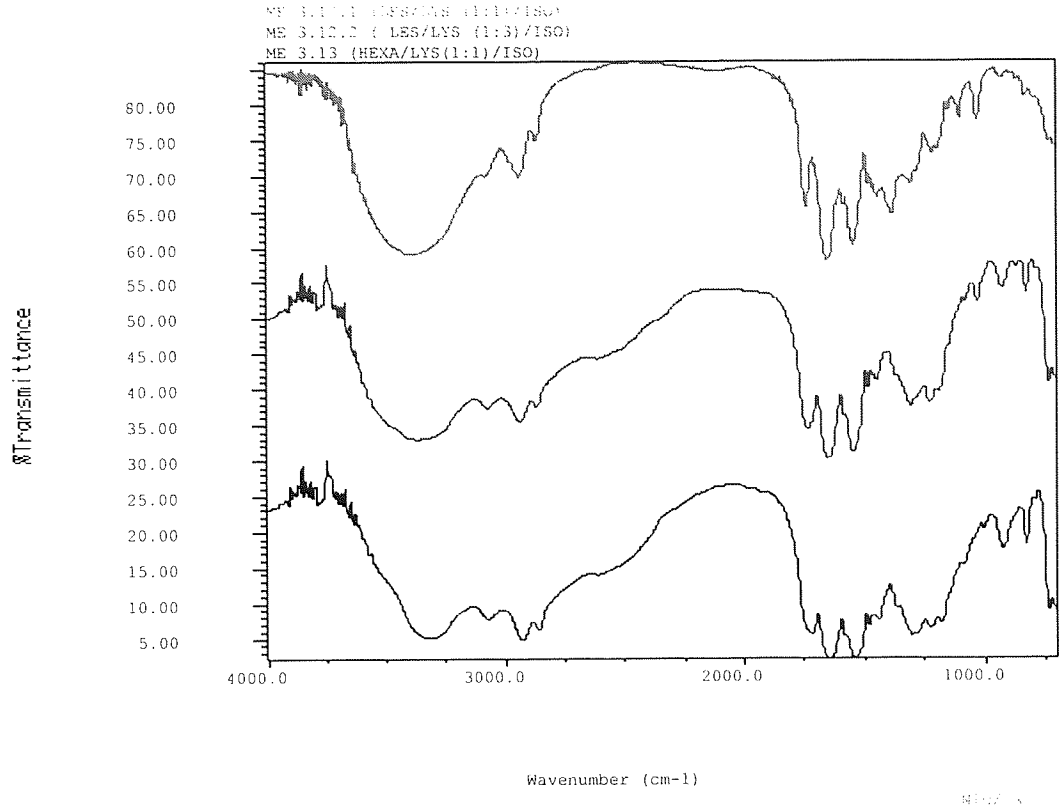
Spectrum 3.



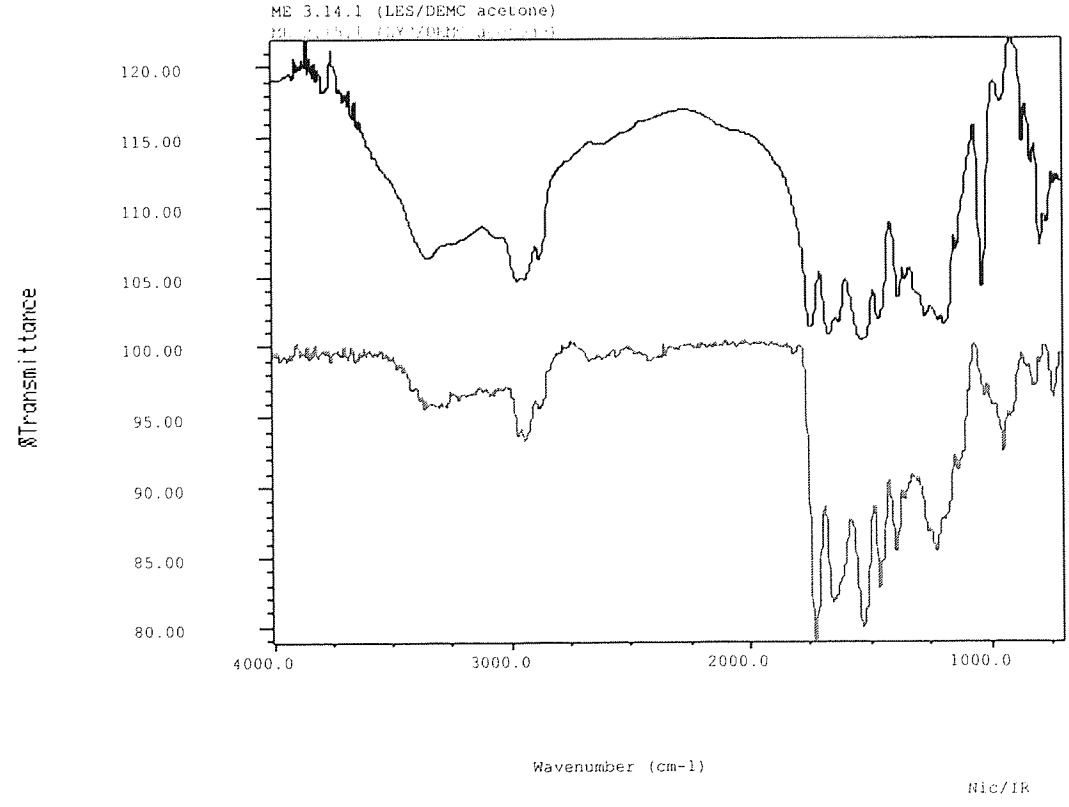
Spectrum 4.



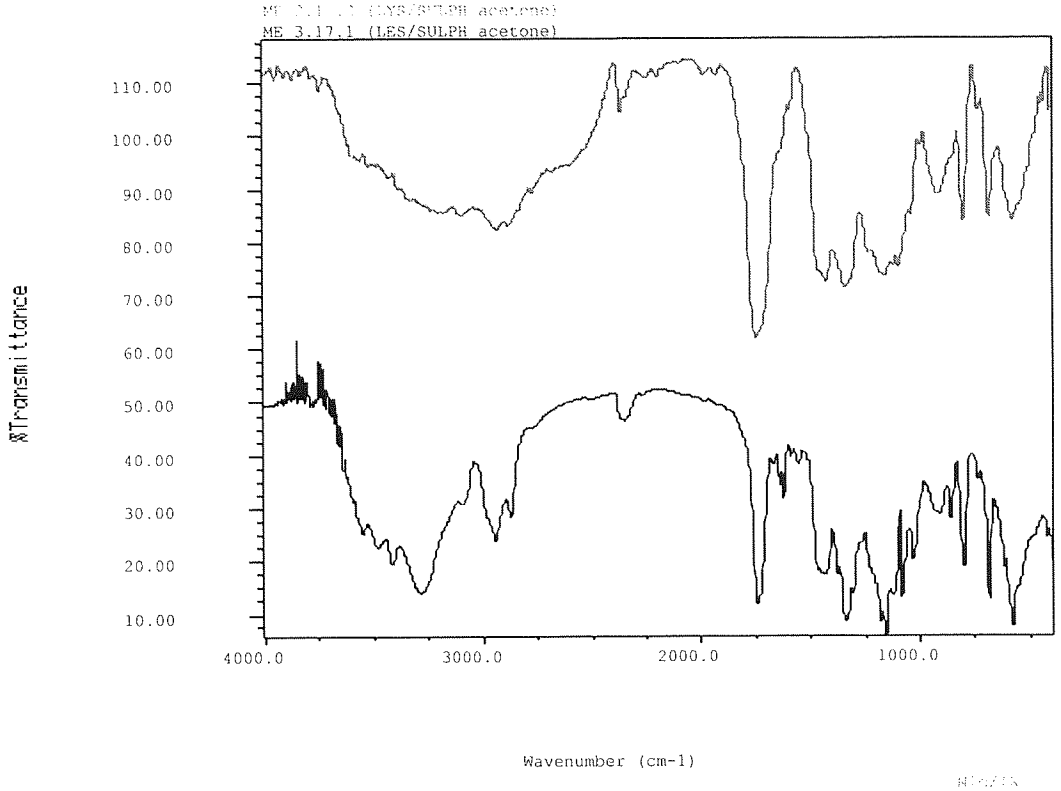
Spectrum 5.



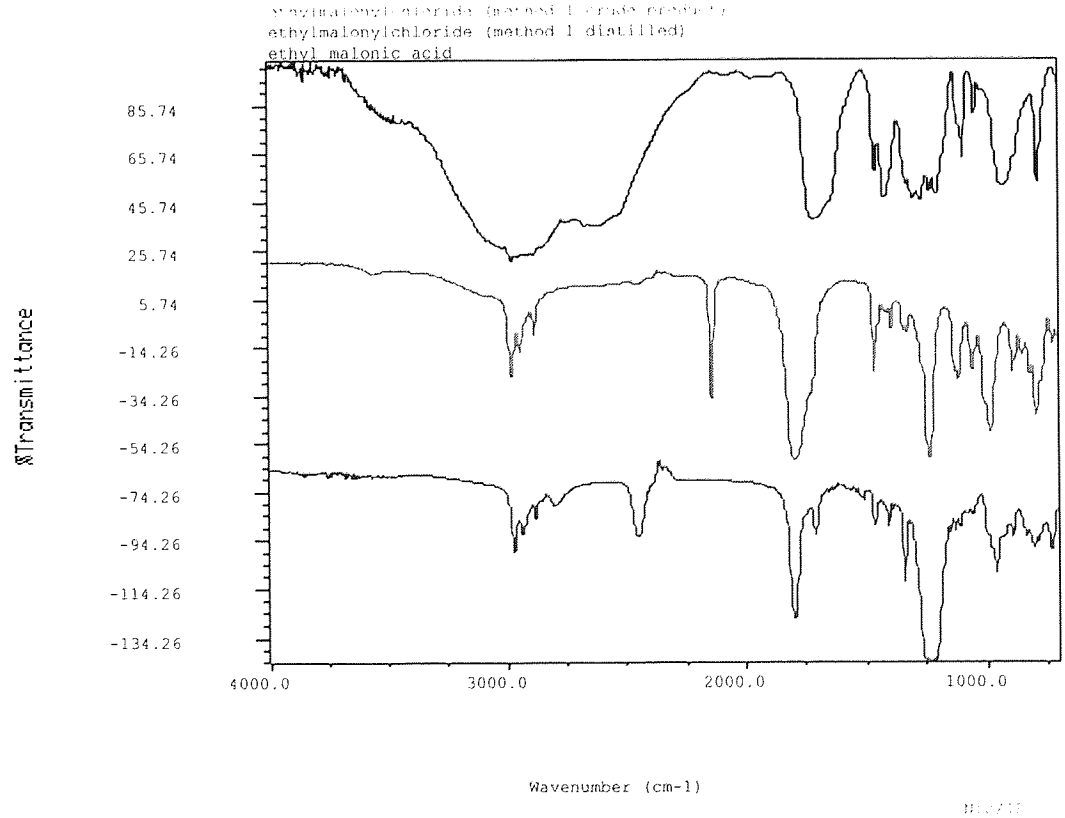
Spectrum 6.



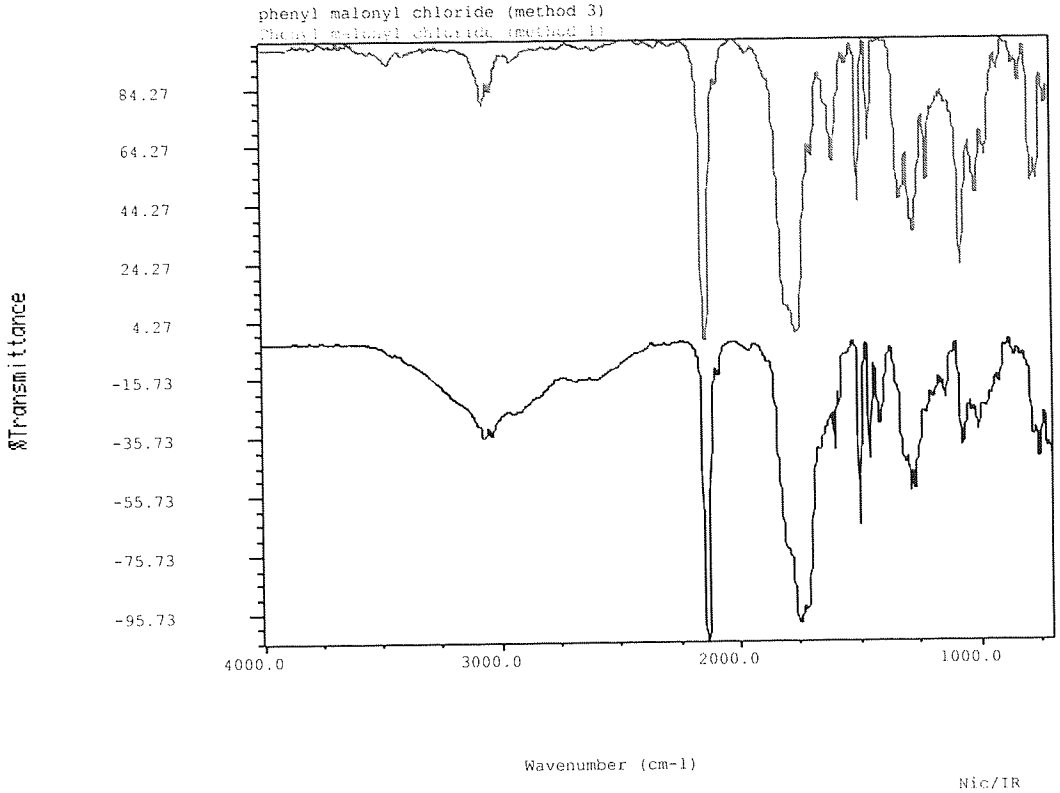
Spectrum 7.



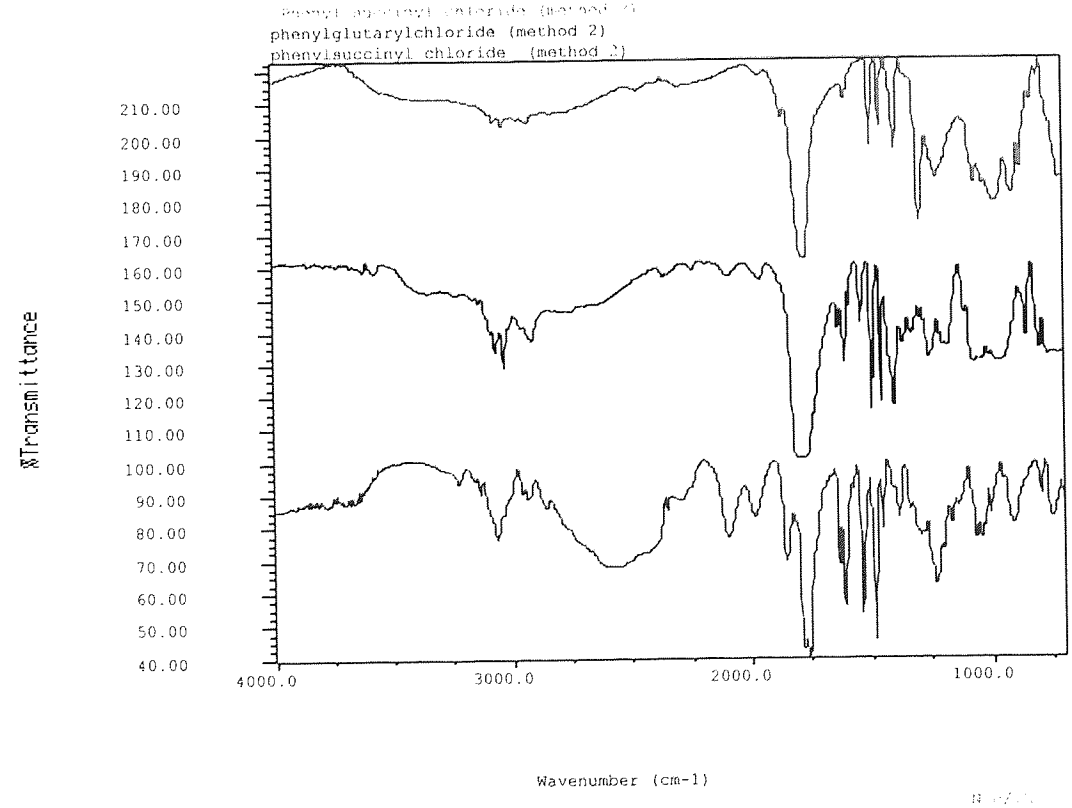
Spectrum 8.



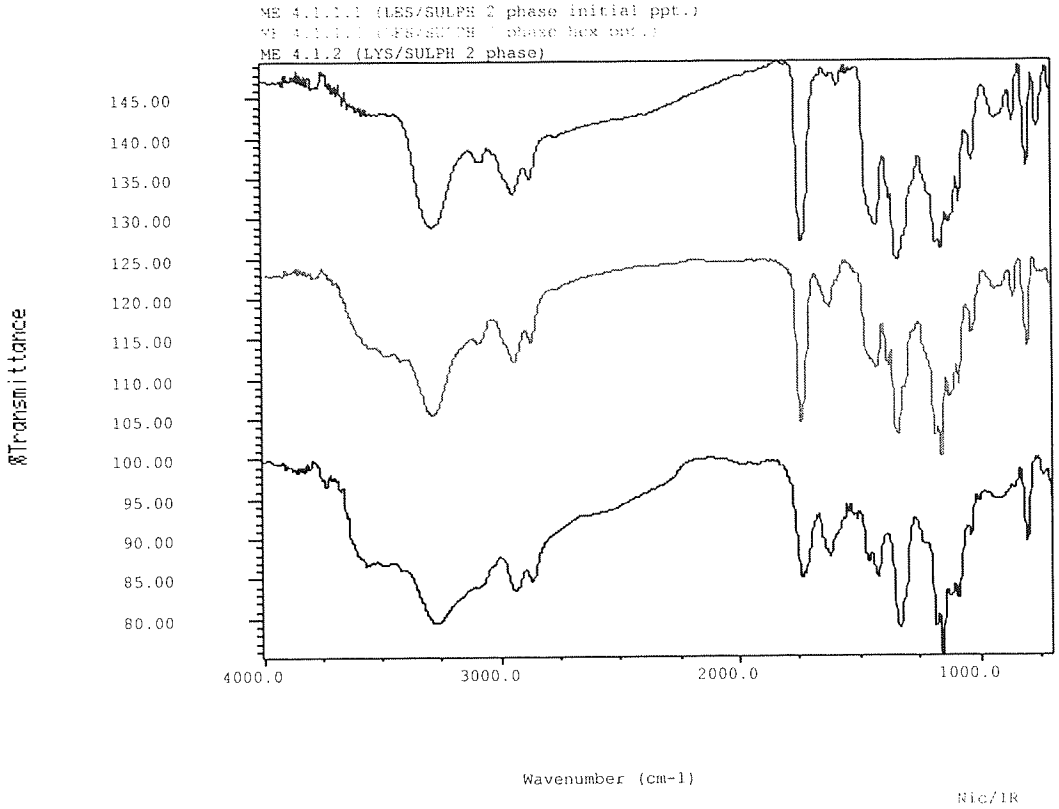
Spectrum 9.



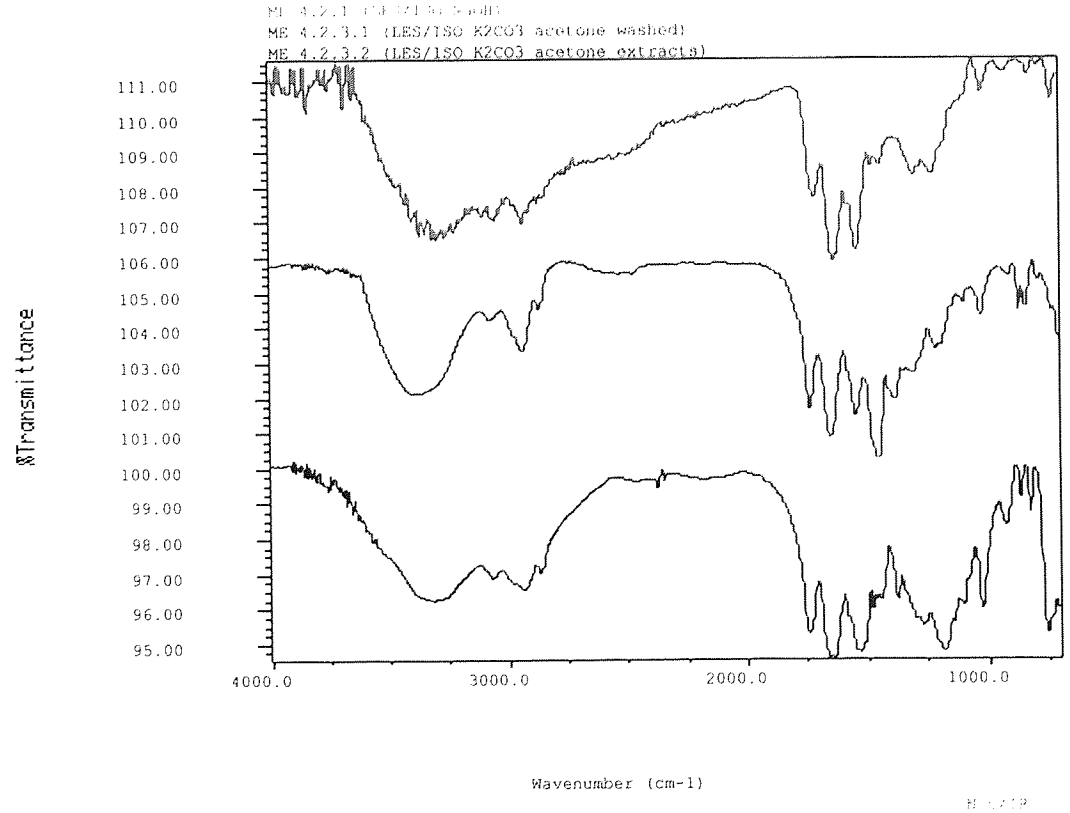
Spectrum 10.



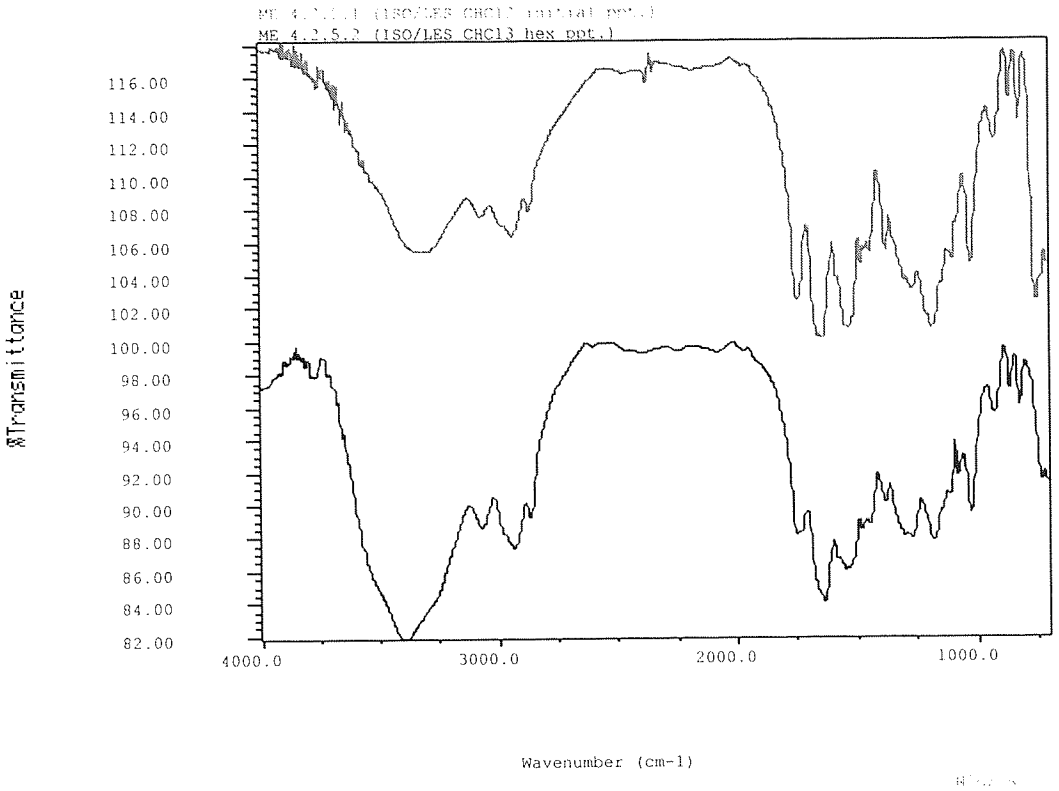
Spectrum 11.



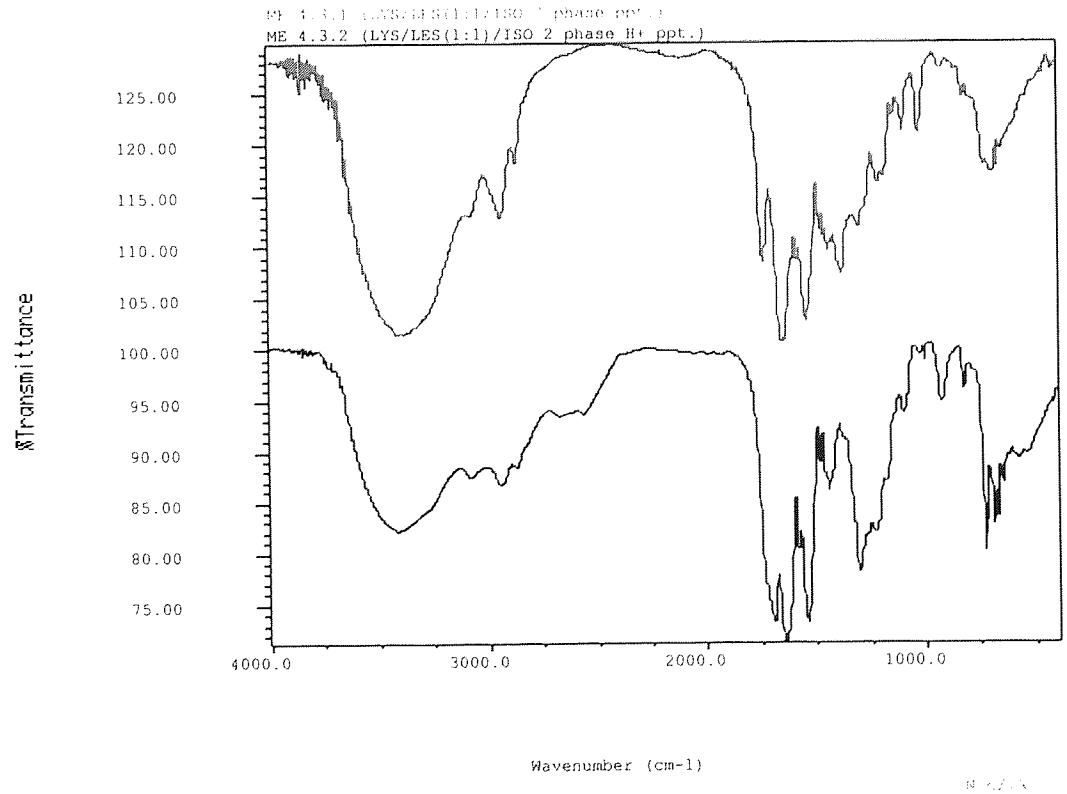
Spectrum 12.



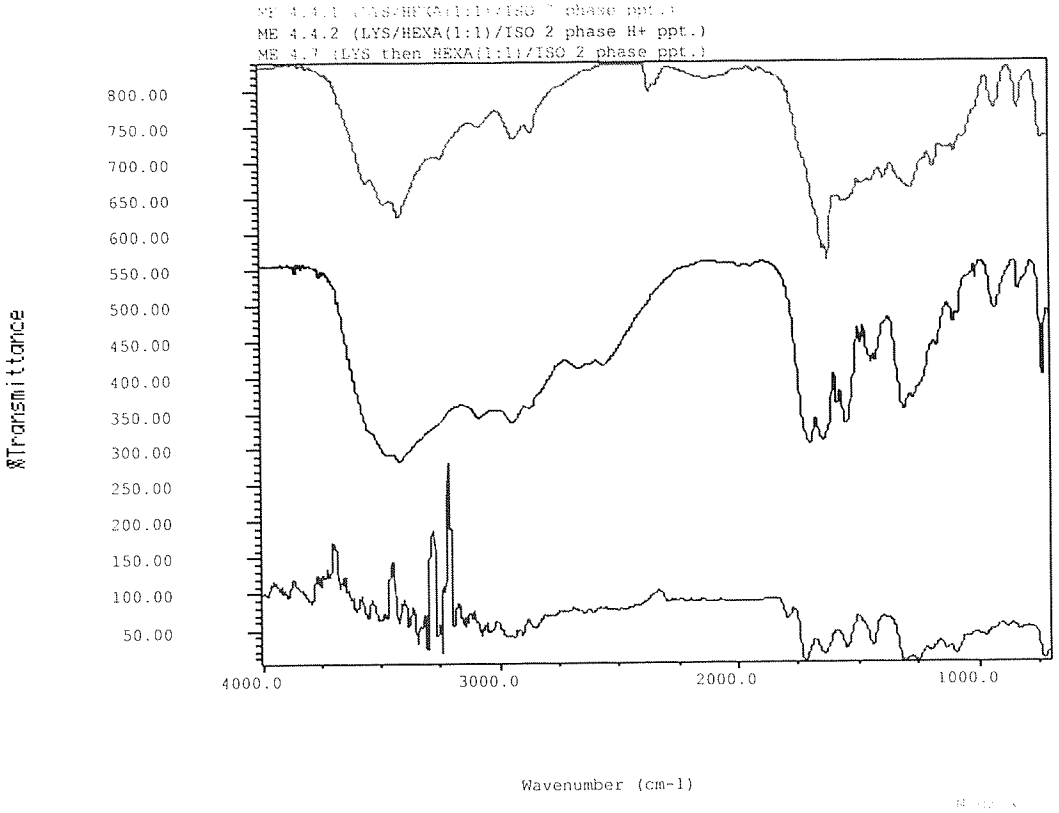
Spectrum 13.



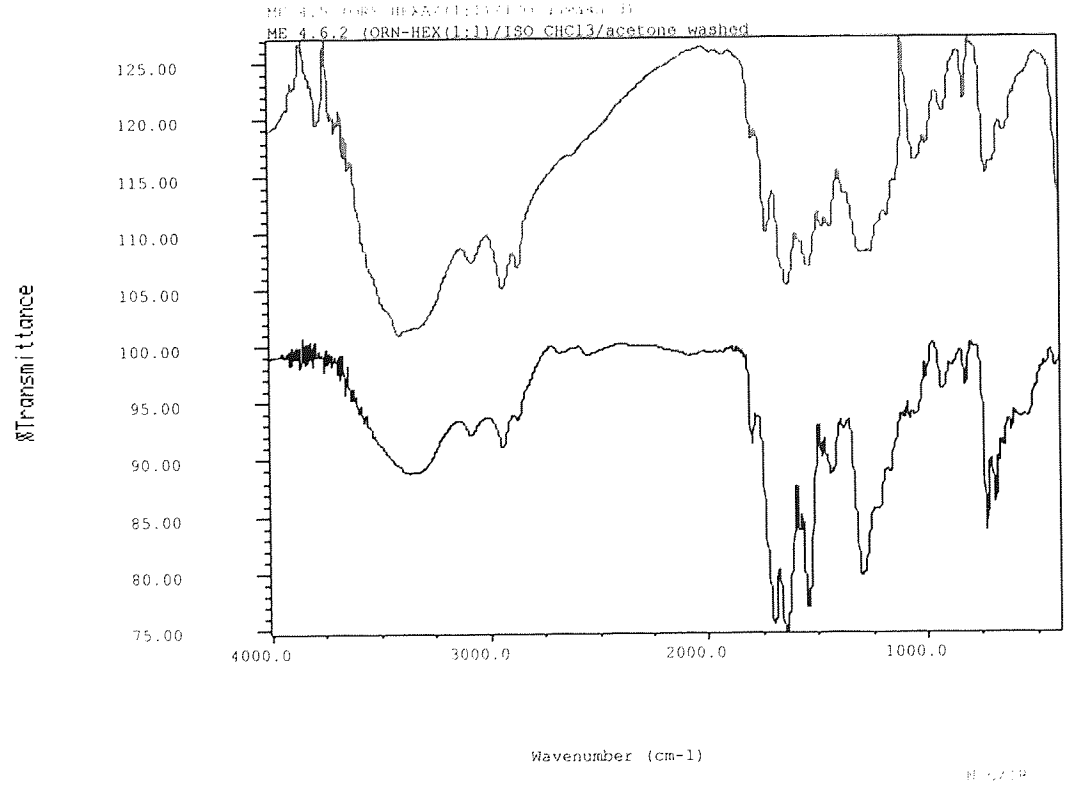
Spectrum 14.



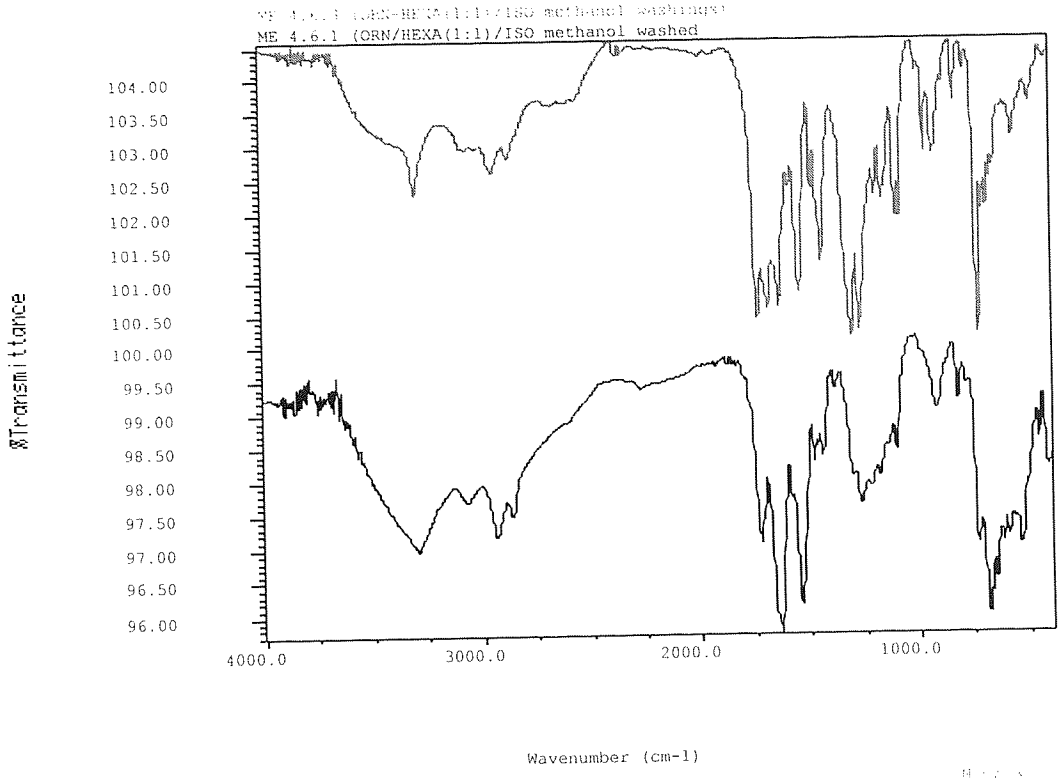
Spectrum 15.



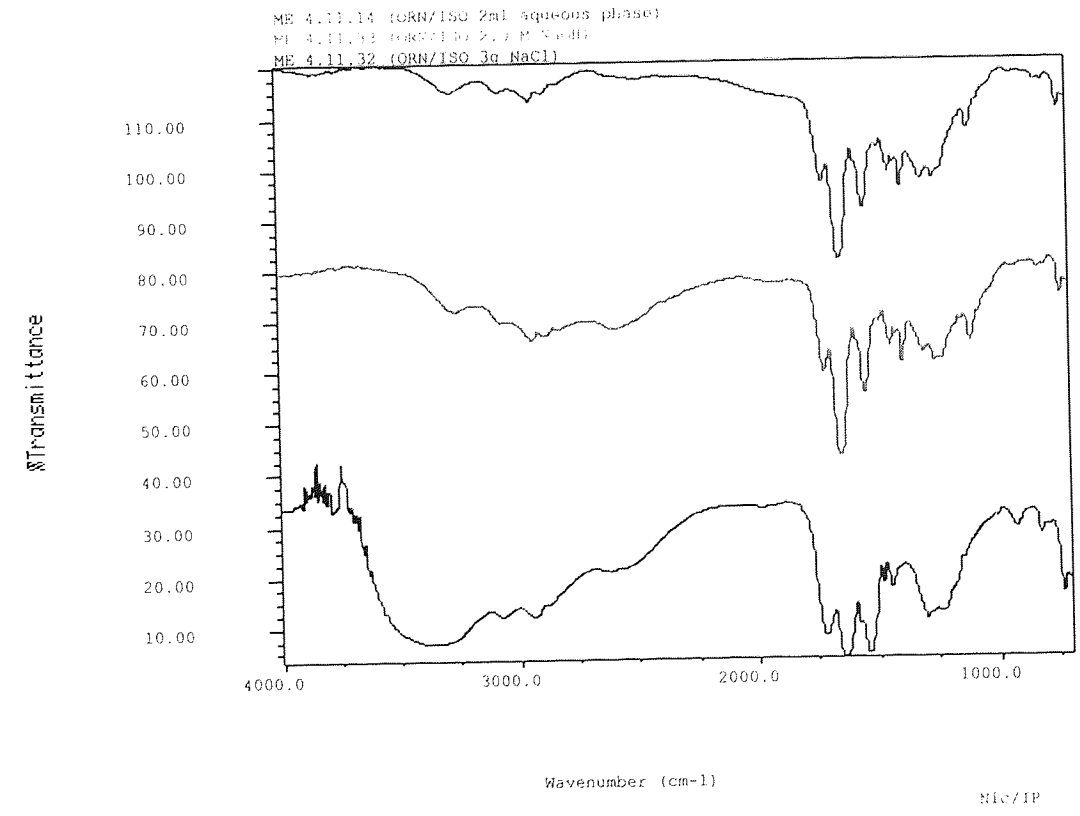
Spectrum 16.



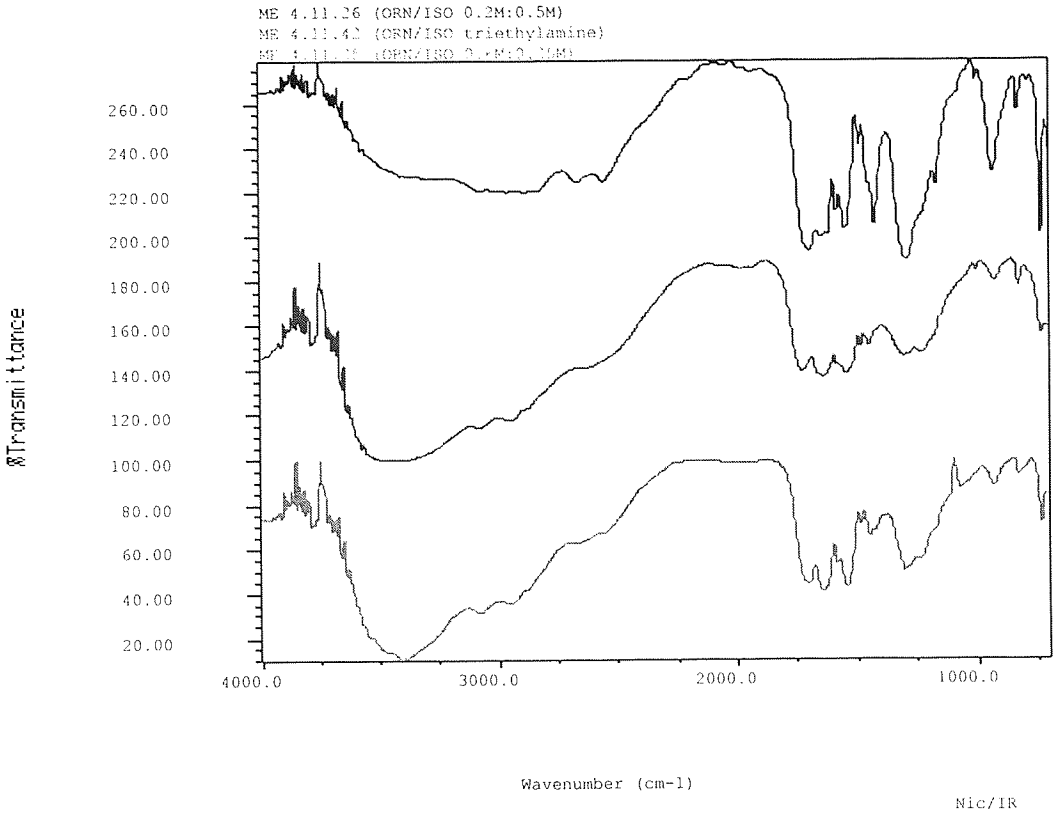
Spectrum 17.



Spectrum 18.



Spectrum 19.



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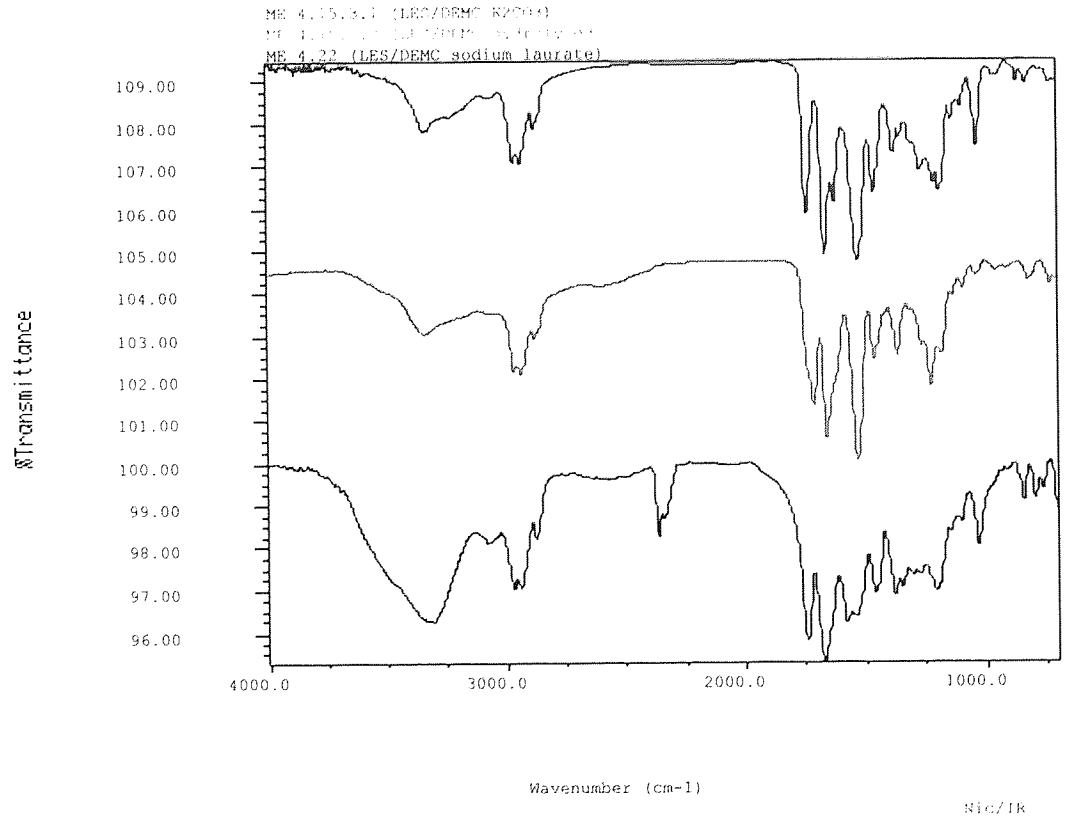

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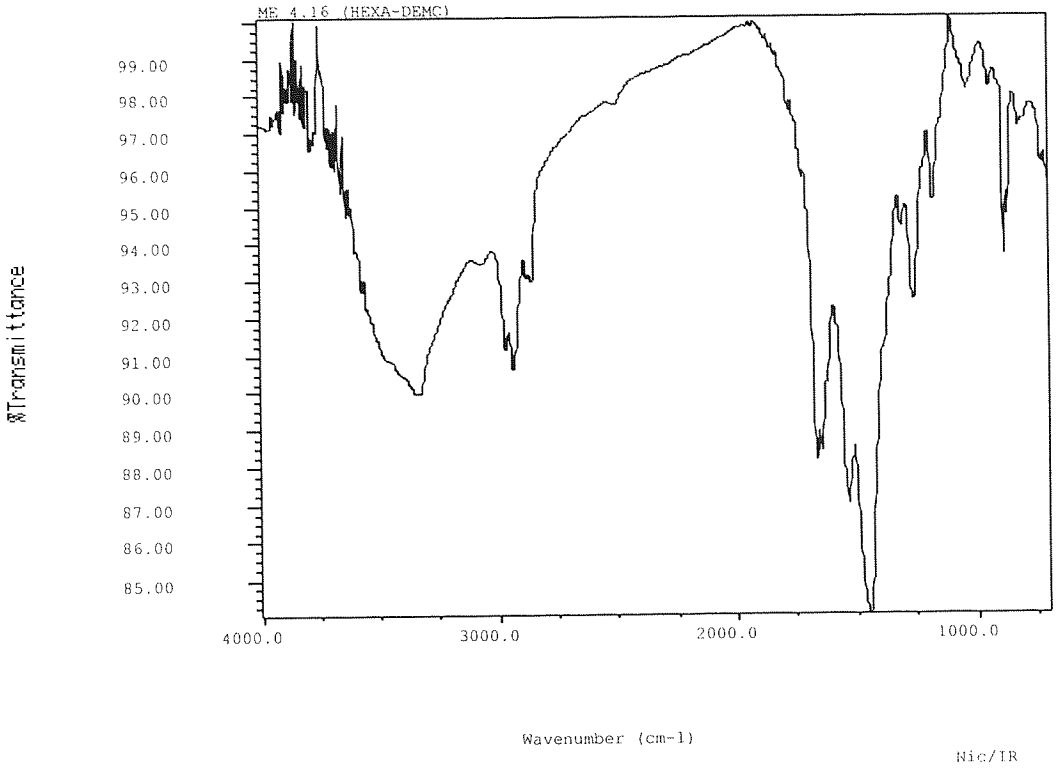

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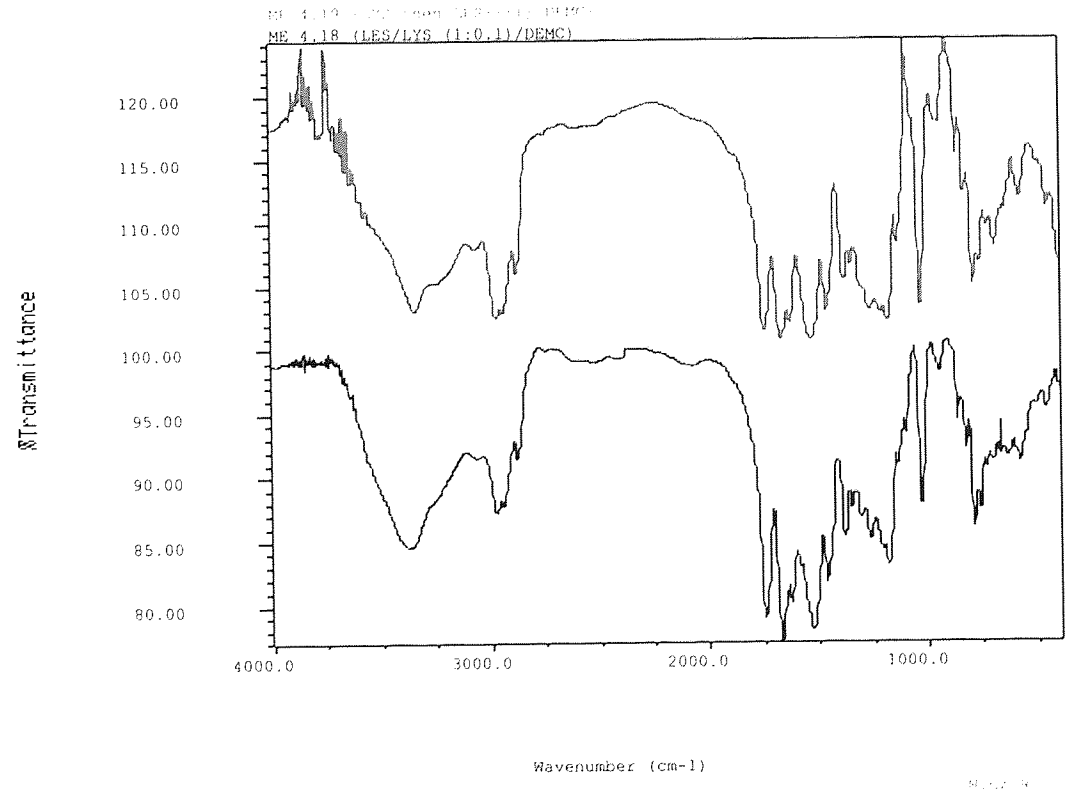

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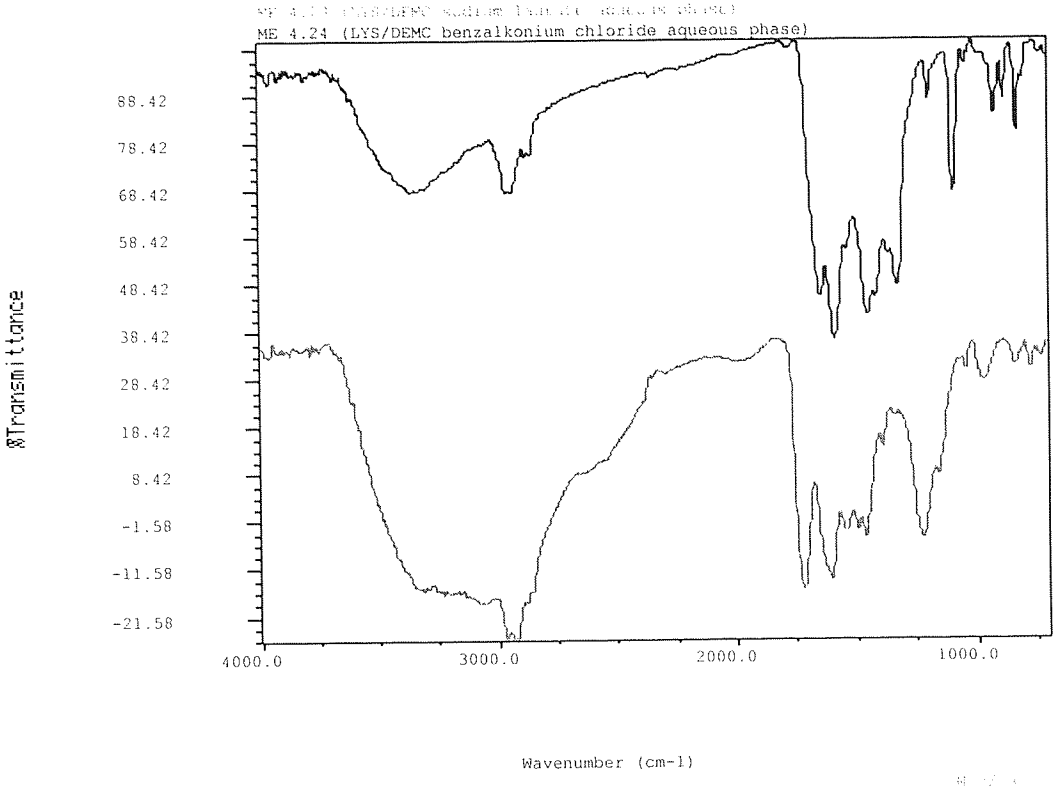

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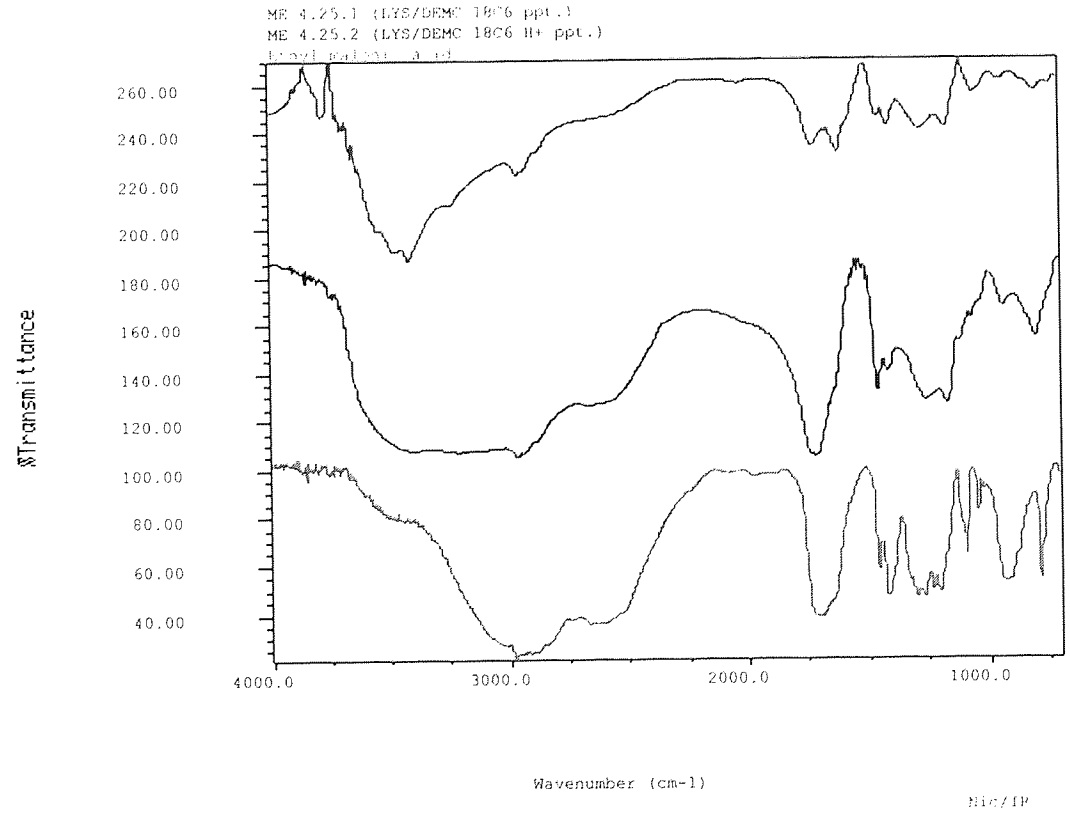

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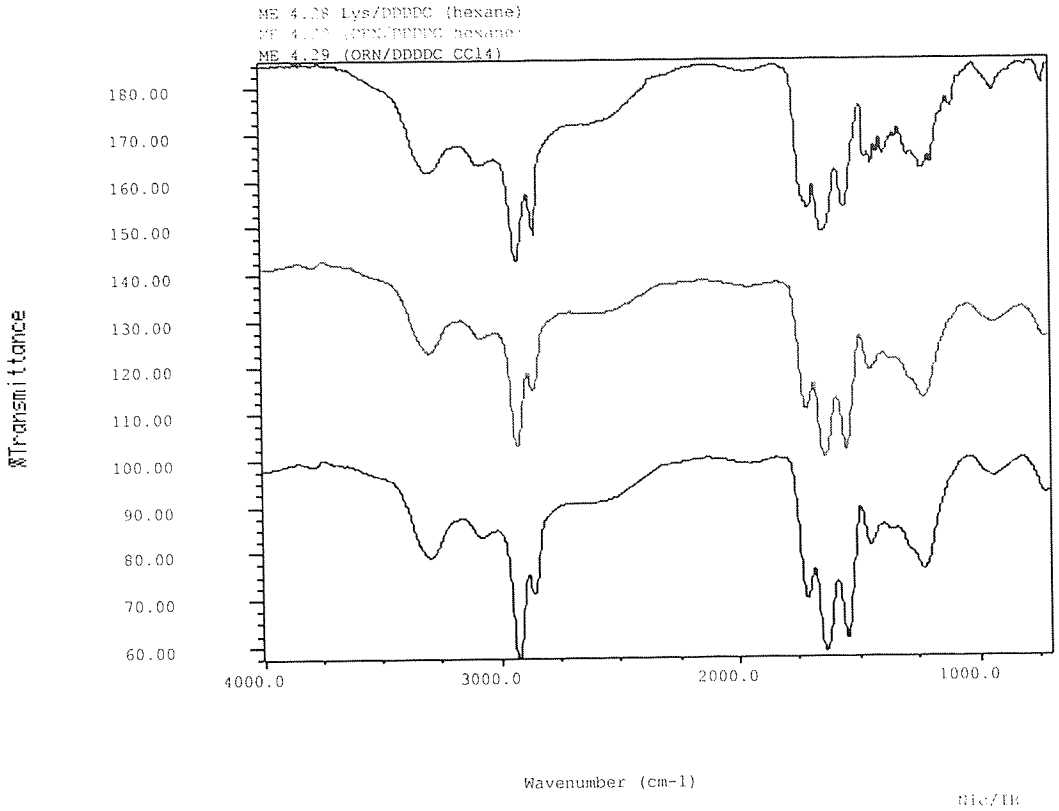

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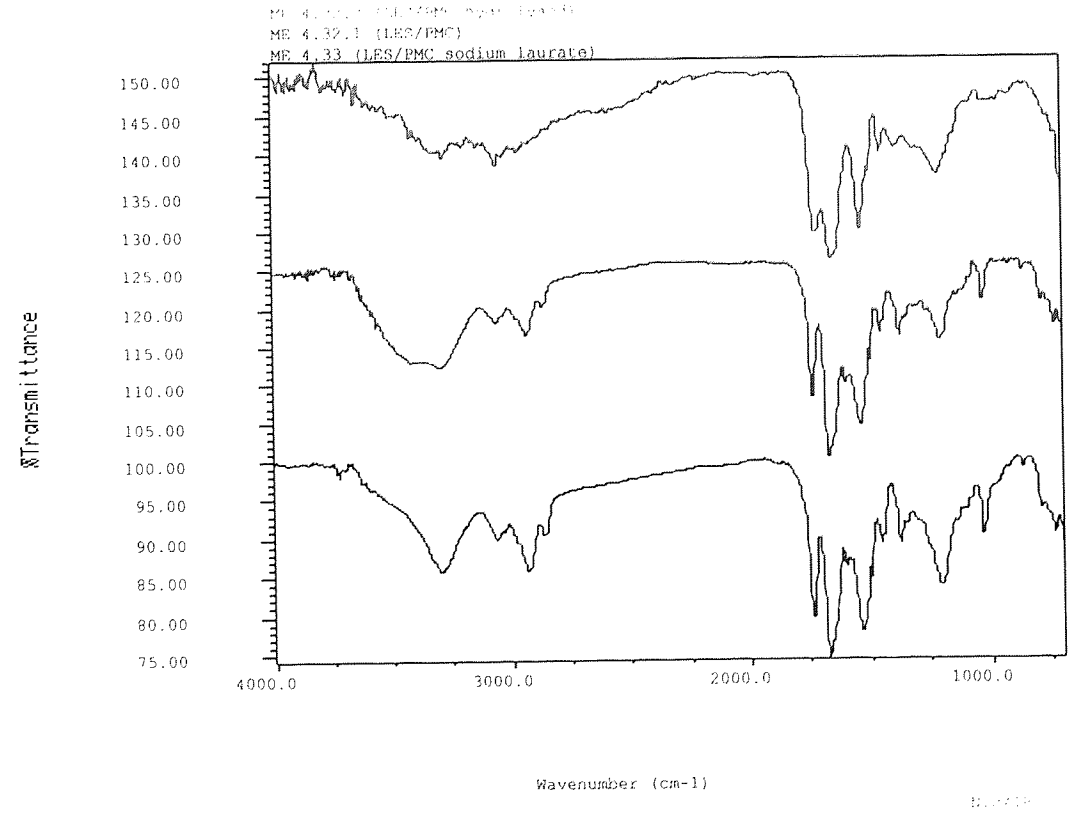

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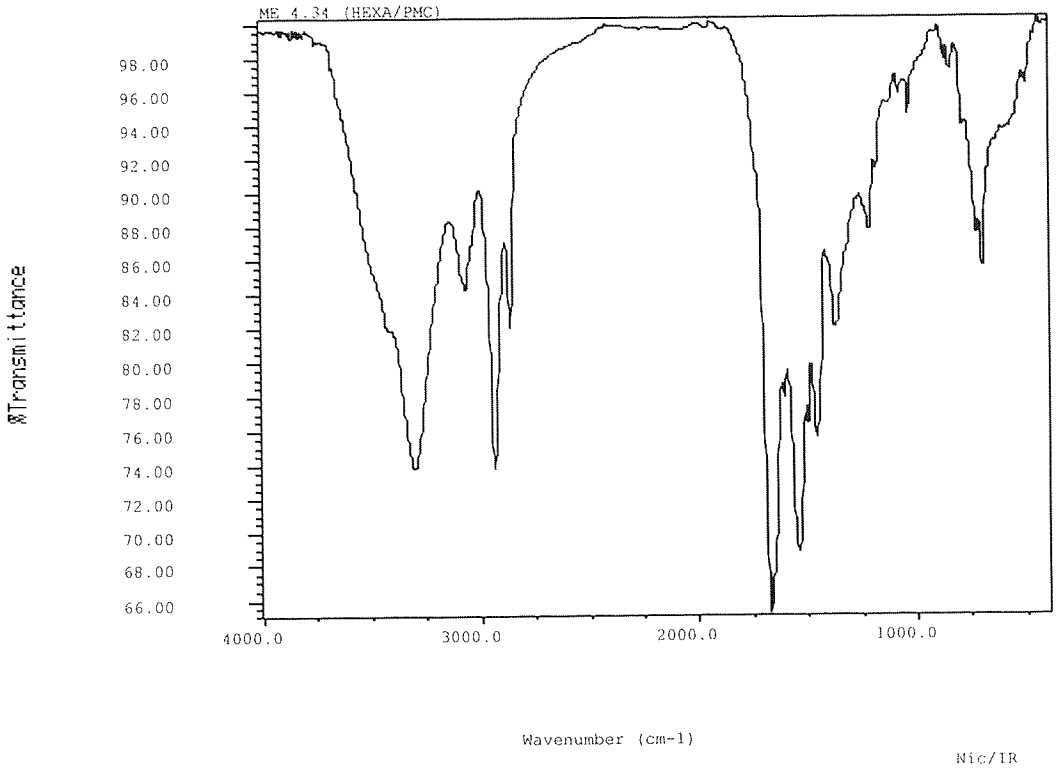

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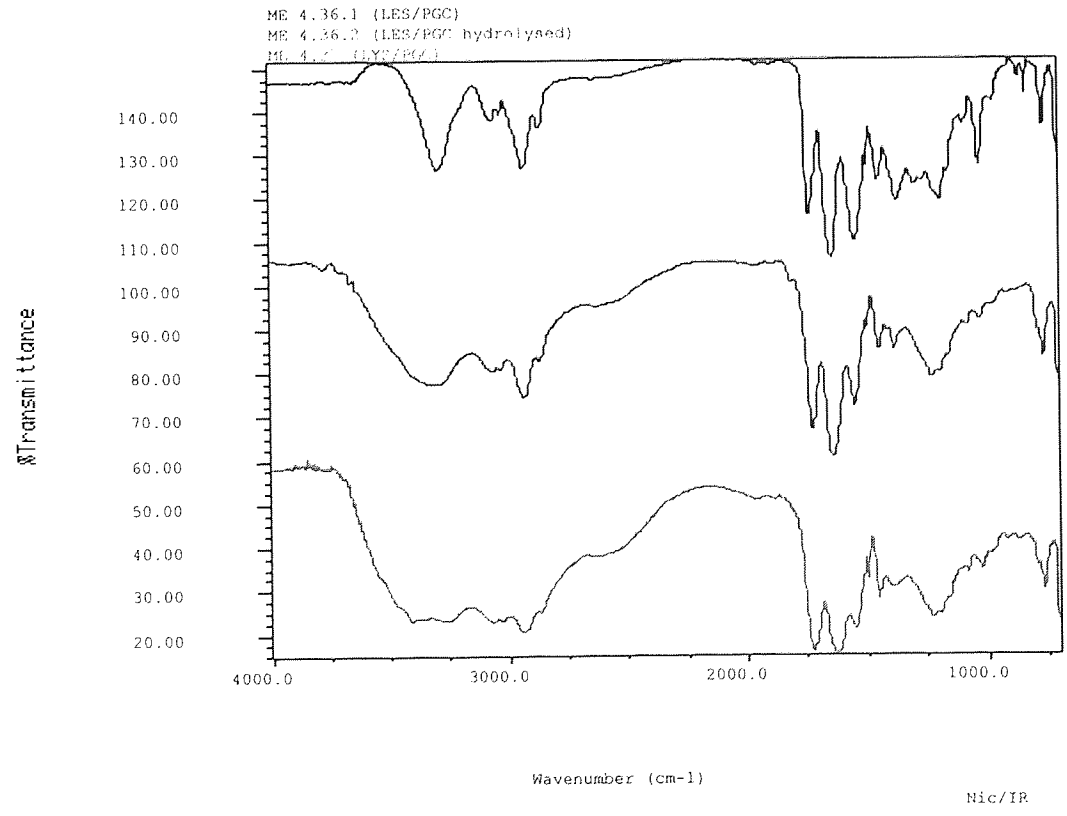

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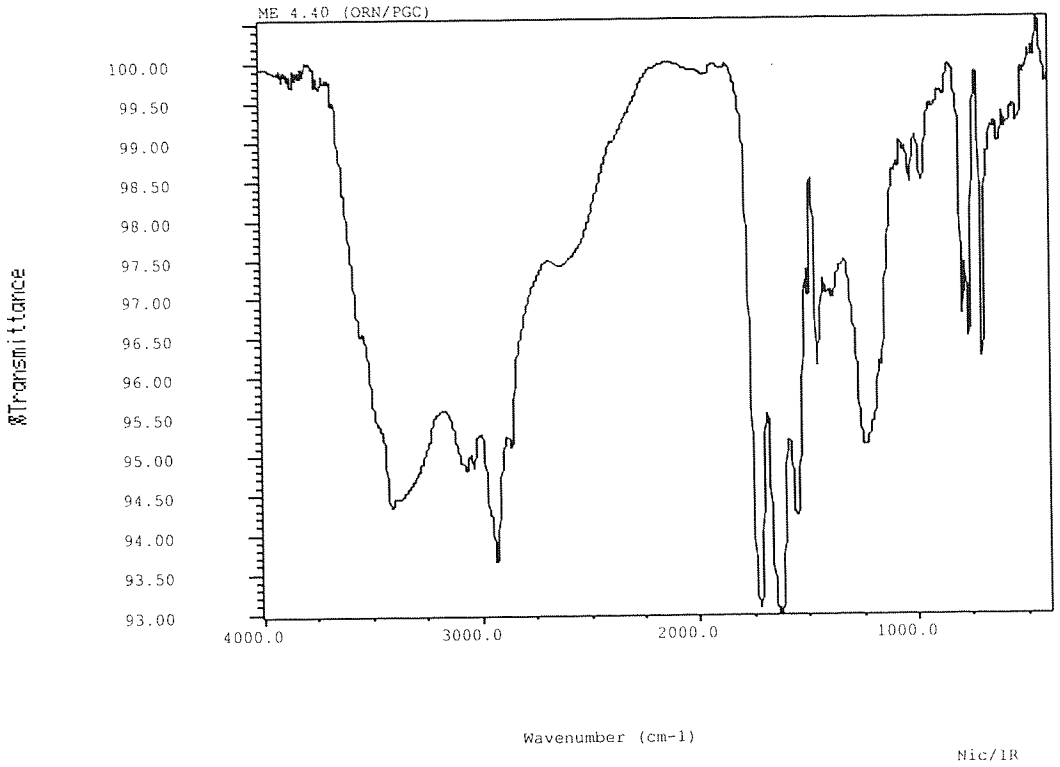

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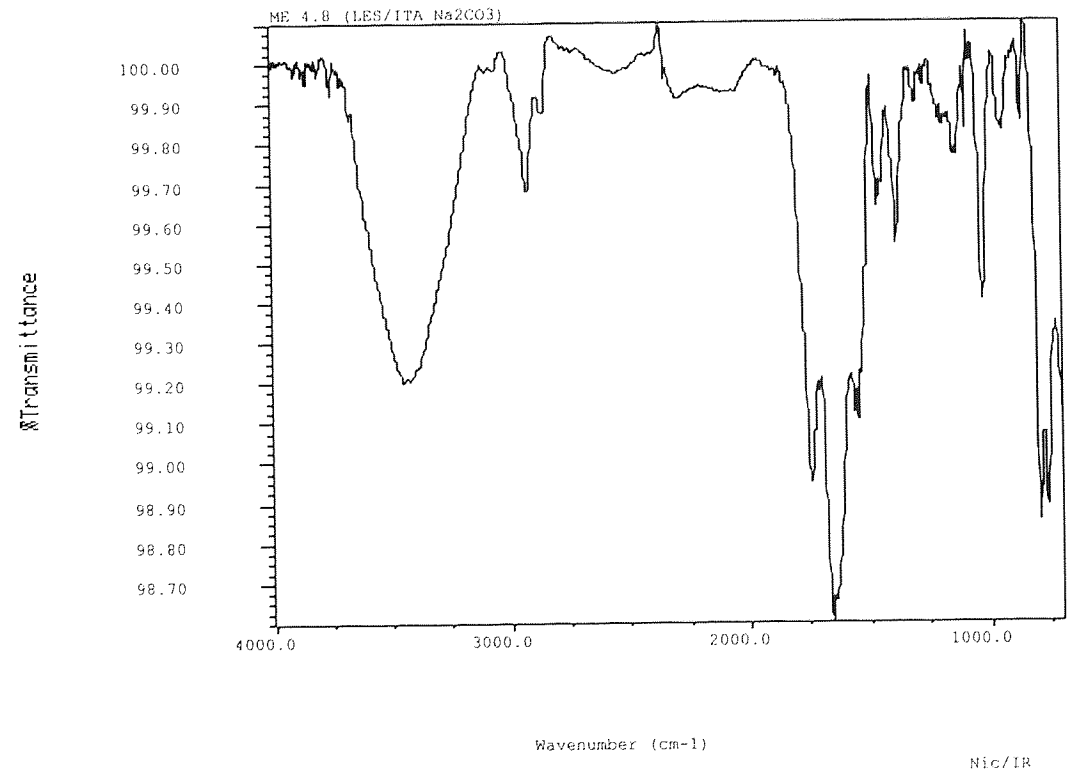

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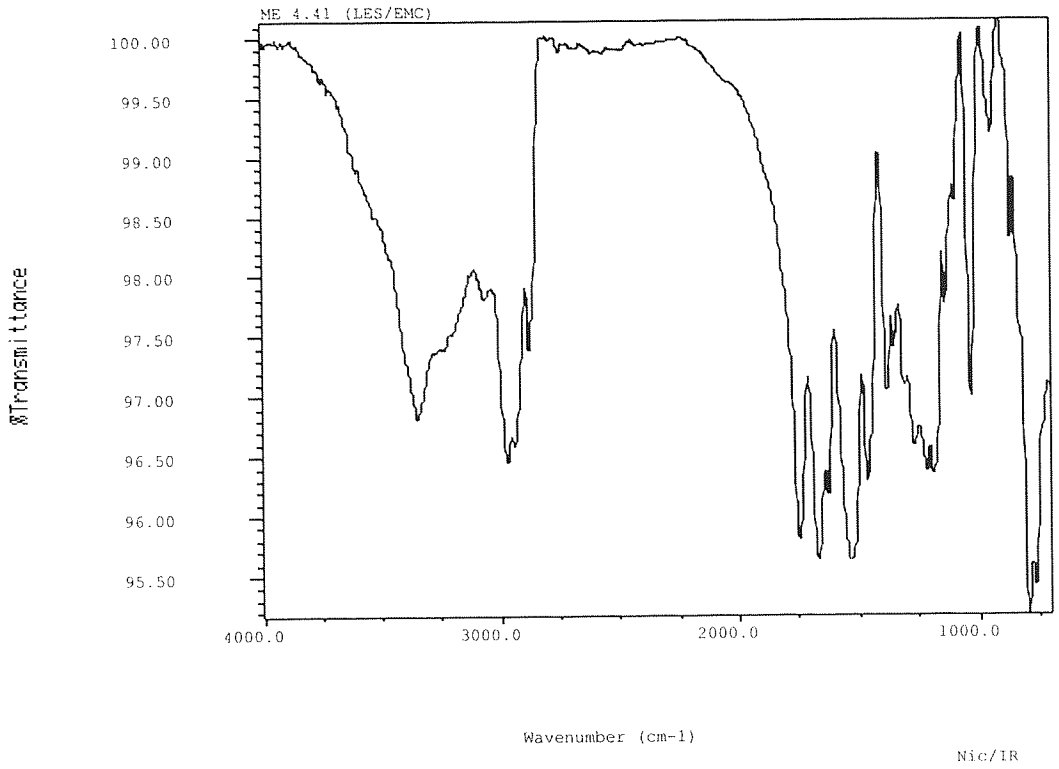
ME 4.41 (LES/EMC)
100.00
99.50
99.00
98.50
98.00
97.50
97.00
96.50
96.00
95.50
%Transmittance
4000.0
3000.0
2000.0
1000.0
Wavenumber (cm-1)
Nic/IR

12. APPENDIX C

SUMMARY OF MECHANICAL PROPERTIES

OF POLY (LYSINE ETHYL ESTER *ISO*-PHTHALAMIDE) FILMS.

Table C1. Mechanical properties of poly (lysine ethyl ester *iso*-phthalamide) films.

Sample	Thickness (mm)	Initial modulus	Tensile strength (MPa)	Elongation at break (%)
0%PCAP	0.23	556	28.5	26.9
	0.23	663	40.3	9.4
	0.24	630	27.3	25.4
	0.26	561	33.8	14.6
	0.27	582	32.7	10.9
	0.31	492	28.1	27.4
Mean	**0.26**	**581**	**31.8**	**19.1**
Standard Deviation	**0.03**	**60**	**4.9**	**8.4**
2% PCAP	0.07	144.1	17.84	68
	0.09	87.2	9.53	214
	0.10	73.7	6.46	178
	0.12	62.1	4.39	106
	0.15	49.7	3.34	75
Mean	**0.11**	**83.3**	**8.31**	**128**
Standard Deviation	**0.03**	**36.7**	**5.82**	**65**

Table C1. Continued.

5% PCAP	0.20	28.14	2.73	78
	0.21	30.67	2.80	311
	0.21	34.10	3.23	90
	0.23	28.99	2.48	443
	0.26	13.49	0.97	608
Mean	**0.23**	**27.08**	**2.44**	**306**
Standard Deviation	**0.03**	**7.93**	**0.87**	**228**
10% PCAP	0.20	6.06	0.12	221
	0.22	5.78	1.04	186
	0.29	6.24	1.03	227
	0.31	4.02	0.52	384
	0.31	4.94	0.62	376
Mean	**0.27**	**5.41**	**0.88**	**279**
Standard Deviation	**0.05**	**0.92**	**0.29**	**94**